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SHORT REPORT

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# Exploring the effect of antenatal depression treatment on children's epigenetic profiles: findings from a pilot randomized controlled trial

Laura S. Bleker<sup>1,2\*</sup> , Jeannette Milgrom<sup>3,4</sup>, Alexandra Sexton-Oates<sup>5</sup>, Tessa J. Roseboom<sup>1,2</sup>, Alan W. Gemmill<sup>3</sup>, Christopher J. Holt<sup>3</sup>, Richard Saffery<sup>5</sup>, Huibert Burger<sup>6</sup> and Susanne R. de Rooij<sup>2</sup>

## Abstract

**Background:** Children prenatally exposed to maternal depression more often show behavioral and emotional problems compared to unexposed children, possibly through epigenetic alterations. Current evidence is largely based on animal and observational human studies. Therefore, evidence from experimental human studies is needed. In this follow-up of a small randomized controlled trial (RCT), DNA-methylation was compared between children of women who had received cognitive behavioral therapy (CBT) for antenatal depression and children of women who had received treatment as usual (TAU). Originally, 54 women were allocated to CBT or TAU. A beneficial treatment effect was found on women's mood symptoms.

**Findings:** We describe DNA methylation findings in buccal swab DNA of the 3–7-year-old children (CBT(N) = 12, TAU(N) = 11), at a genome-wide level at 770,668 CpG sites and at 729 CpG sites spanning 16 a priori selected candidate genes, including the glucocorticoid receptor (*NR3C1*). We additionally explored associations with women's baseline depression and anxiety symptoms and offspring DNA methylation, regardless of treatment. Children from the CBT group had overall lower DNA methylation compared to children from the TAU group (mean  $\Delta\beta = -0.028$ , 95% CI  $-0.035$  to  $-0.022$ ). Although 68% of the promoter-associated *NR3C1* probes were less methylated in the CBT group, with cg26464411 as top most differentially methylated CpG site ( $p = 0.038$ ), mean DNA methylation of all *NR3C1* promoter-associated probes did not differ significantly between the CBT and TAU groups (mean  $\Delta\beta = 0.002$ , 95%CI  $-0.010$  to  $0.011$ ). None of the effects survived correction for multiple testing. There were no differences in mean DNA methylation between the children born to women with more severe depression or anxiety compared to children born to women with mild symptoms of depression or anxiety at baseline (mean  $\Delta\beta$  (depression) =  $0.0008$ , 95% CI  $-0.007$  to  $0.008$ ; mean  $\Delta\beta$  (anxiety) =  $0.0002$ , 95% CI  $-0.004$  to  $0.005$ ).

**Conclusion:** We found preliminary evidence of a possible effect of CBT during pregnancy on widespread methylation in children's genomes and a trend toward lower methylation of a CpG site previously shown by others to be linked to depression and child maltreatment. However, none of the effects survived correction for multiple testing and larger studies are warranted.

**Trial registration:** Trial registration of the original RCT: [ACTRN12607000397415](https://www.actr.org.au/actr12607000397415). Registered on 2 August 2007.

**Keywords:** DNA methylation, Epigenetics, Neurodevelopment, Antenatal depression, CBT, Programming

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## Background

Many pregnant women experience clinically significant depressive symptoms before delivery, with an estimated prevalence of 7.4 to 12.8% [1]. Mounting evidence demonstrates that children prenatally exposed to maternal depression more often have a difficult temperament [2], are more prone to develop internalizing and externalizing behavioral problems [3–7], show poorer performance on cognitive tasks [8, 9], and more often develop depression and anxiety symptoms themselves in (pre)adolescence [10–12]. One mechanism by which antenatal depression might influence susceptibility for psychopathology is by epigenetic regulation of gene expression [13, 14]. Epigenetic mechanisms regulate the activity of DNA and include post-translational histone modification, micro-RNAs, and DNA methylation [15]. In contrast to the fixed genotype, the epigenome has shown to be highly variable early in development under the influence of environmental factors [16, 17].

Animal studies have provided evidence that antenatal stress alters methylation of offspring genes involved in neurodevelopment and is associated with behavioral changes. For example, exposure to chronic stress in early gestation in mice resulted in a stress-sensitive phenotype in male offspring, showing increased immobility in the tail suspension and forced swim test and heightened hypothalamic pituitary adrenal (HPA) axis responsivity, which was accompanied by increased DNA methylation and decreased gene expression of the glucocorticoid receptor in the hippocampus and amygdala [18]. Moreover, alterations in epigenetic profiles have been shown to remain stable across generations, passing on susceptibility for emotional and behavioral disorders from one generation to the next [19].

Since 2008, many human studies have investigated associations between prenatal stress exposure and offspring gene methylation, with a special focus on *NR3C1*, coding for the glucocorticoid receptor [20]. While the reported effect sizes are usually small, increased methylation status of *NR3C1* has been linked to an increased HPA axis stress-response [21]. All studies to date are, however, observational and therefore susceptible to confounding by factors that are both associated with antenatal stress and with methylation patterns, such as maternal smoking during pregnancy [22]. Experimental designs including follow-up of children are currently scarce and urgently needed to establish causality between intrauterine exposures and later life outcomes [23].

The current study investigated effects of maternal depression treatment during pregnancy on DNA methylation profiles in the children. In the Beating the Blues before Birth (BBB) study, pregnant women with a confirmed Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) depressive disorder were randomized to either the intervention group, consisting of eight cognitive behavioral therapy

(CBT) sessions, or to a control group, consisting of treatment as usual (TAU), which comprised case-managing by a midwife or referral to a general practitioner. Beneficial treatment effects favoring the intervention were found on maternal depression and anxiety. Anxiety symptoms significantly decreased, and depressive symptoms showed a decreasing trend nearly reaching significance, in the intervention versus the control group [24].

We hypothesized that compared to the control group, the intervention would be associated with a change in DNA methylation profiles of buccal swab DNA from the children, (1) at an epigenome-wide level, (2) at 16 a priori selected candidate genes, and (3) at promoter-associated glucocorticoid receptor (*NR3C1*) probes. We additionally explored whether severity of maternal symptoms of depression and anxiety at baseline would be associated with DNA methylation profiles in the children, regardless of treatment.

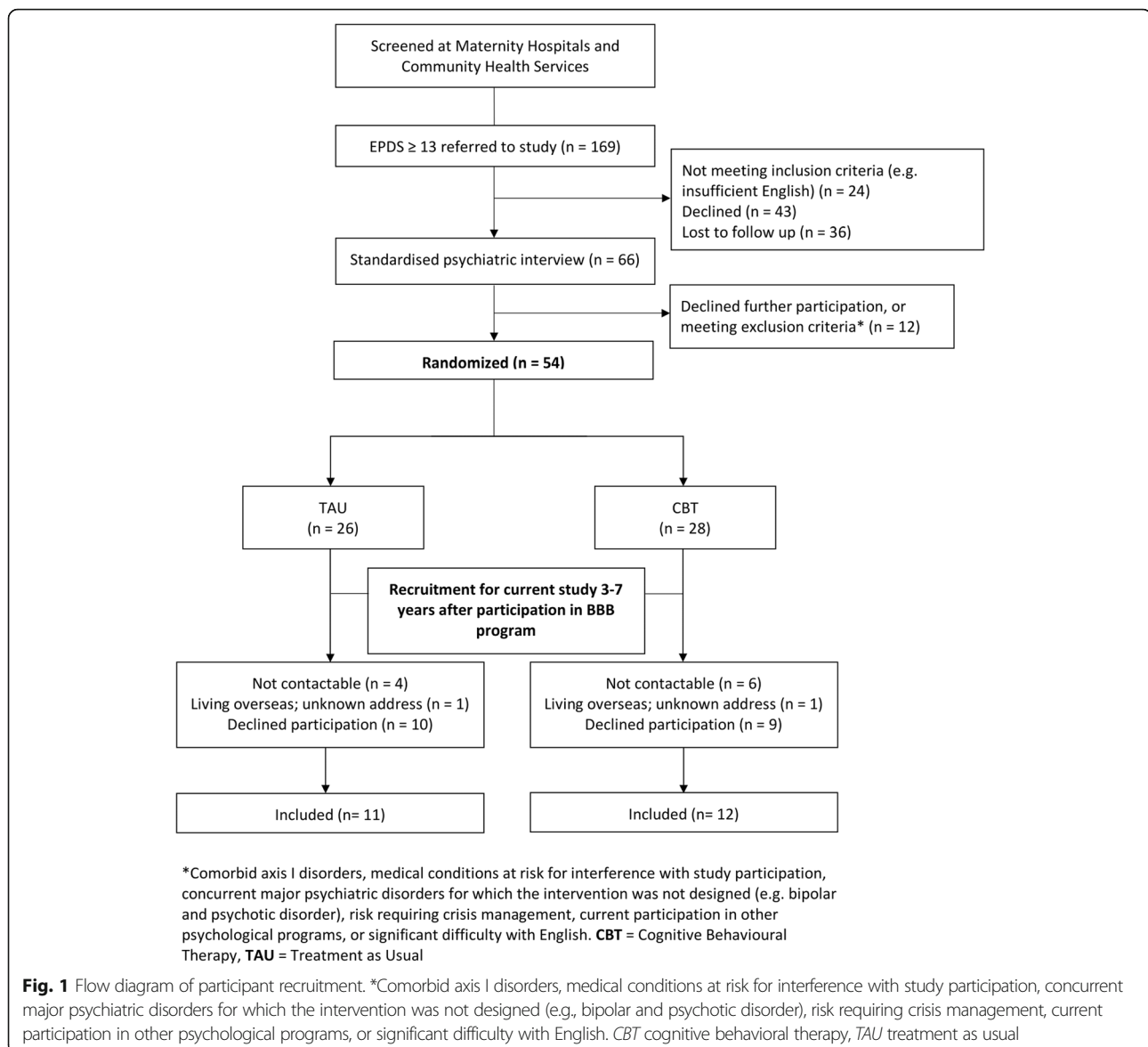
## Results

### Study sample characteristics

Of the original study group of 54 women, 2 women had moved overseas to unknown addresses, and 10 women could not be traced. This resulted in 42 women being invited to participate in the current study. In total, 19 women declined to participate. Reasons for declining were lack of time, a lack of interest in being involved, or not wanting their child's DNA to be used for study purposes. This resulted in a study group of 23 women and their children who agreed to participate in the current study, 12 (42.9%) women from the intervention group and 11 (42.3%) women from the control group (flowchart; Fig. 1). Table 1 shows baseline characteristics of all women from the original study, women that did not participate, and women that did participate in the current follow-up. In the intervention and control group alike, women that responded to the current follow-up had lower Beck Depression Inventory (BDI-II) and lower Beck Anxiety Inventory (BAI) scores, less often reported using antidepressants, and were more highly educated with a higher annual income compared to non-responders at baseline. In the intervention group, participating women were more often born in Australia and married compared to women who did not participate, whereas in the control group, women were less often born in Australia and married compared to non-responders. Current demographics of the women and their children are shown in Table 2. Less women from the intervention group were currently using an antidepressant, their income was higher, and they more often drank one or more alcoholic unit per week, as compared to the control group.

### Association between genome-wide DNA methylation and allocation

Linear regression analysis was used to identify specific differentially methylated probes according to allocation.



This took into account variation associated with the following covariates: birth weight, HM850 array chip position, sex and age, as identified by principal component analysis (PCA). Linear regression analysis revealed a total of 4780 differentially methylated probes at a nominal significance level ( $p < 0.01$ , uncorrected for multiple testing) between the intervention and the control group, showing higher DNA methylation in the control group (mean  $\Delta\beta = -0.028$ , 95% CI  $-0.035$  to  $-0.022$ ,  $p < 0.001$ ). Adding current income as an additional covariate did not significantly alter the results (mean  $\Delta\beta = -0.026$ , 95% CI  $-0.031$  to  $-0.021$ ,  $p < 0.001$ ). The top 100 differentially methylated probes are presented in Table 3 of the Appendix. Table 4 shows the ten most differentially methylated probes. Of the top five differentially methylated probes, three probes with annotated genes were probe

cg15495292 on the *AIG1* gene (uncorrected  $p = 4.01E-06$ , corrected  $p = 0.999$ ), cg05155812 on the *SUN1* gene (uncorrected  $p = 1.56E-05$ , corrected  $p = 0.999$ ), and cg18818484 on the *PTCHD2* gene (uncorrected  $p = 2.20E-05$ , corrected  $p = 0.999$ ). After correcting for multiple testing (corrected  $p \leq 0.01$ ), no probes remained significantly associated with the intervention.

#### Candidate gene-specific DNA methylation and allocation

In addition to an exploratory genome-wide analysis (above), we also tested for associations with a list of a priori chosen candidate genes. Table 5 shows the results of the unpaired Mann-Whitney-Wilcoxon tests, comparing mean DNA methylation of 16 candidate genes between the intervention and control group. No genes were significantly differentially methylated at a nominal

**Table 1** Baseline characteristics of all participants in a trial evaluating an antenatal cognitive behavioral therapy (CBT) versus treatment as usual (TAU), those that responded, and those that did not respond to the 5-year follow-up

|   | All participants     |                      | Not participating in 5-year follow-up |                      | Participating in 5-year follow-up |                      |
|---|----------------------|----------------------|---------------------------------------|----------------------|-----------------------------------|----------------------|
|   | CBT ( <i>n</i> = 28) | TAU ( <i>n</i> = 26) | CBT ( <i>n</i> = 16)                  | TAU ( <i>n</i> = 15) | CBT ( <i>n</i> = 12)              | TAU ( <i>n</i> = 11) |
| Baseline demographics   |                      |                      |                                       |                      |                                   |                      |
| Mean (SD) BDI pre-treatment score                             | 30.8 (9.5)           | 30.5 (8.9)           | 31.6 (9.7)                            | 31.2 (7.8)           | 29.6 (9.5)                        | 29.5 (10.4)          |
| Mean (SD) BAI pre-treatment score                             | 22.8 (10.0)          | 21.2 (10.2)          | 25.4 (10.1)                           | 22.8 (12.2)          | 19.2 (9.0)                        | 19.3 (7.1)           |
| Mean (SD) BDI post-treatment score                            | 13.0 (9.8)           | 17.4 (9.8)           | 12.9 (10.1)                           | 17.3 (10.8)          | 13.0 (10.0)                       | 17.6 (9.0)           |
| Mean (SD) BAI post-treatment score                            | 10.6 (7.6)           | 16.7 (11.8)          | 9.6 (5.4)                             | 17.6 (14.3)          | 11.6 (9.9)                        | 15.3 (7.1)           |
| Mean (SD) $\Delta$ BDI score (post-treatment – pre-treatment) | – 18.6 (10.0)        | – 13.2 (12.8)        | – 20.4 (12.0)                         | – 14.5 (10.4)        | – 16.6 (7.3)                      | – 11.5 (16.1)        |
| Mean (SD) $\Delta$ BAI score (post-treatment – pre-treatment) | – 11.2 (9.4)         | – 4.3 (8.3)          | – 14.5 (10.1)                         | – 5.0 (9.8)          | – 7.5 (7.2)                       | – 3.1 (6.0)          |
| Mean (SD) maternal age in years                               | 32.9 (5.9)           | 31.0 (5.8)           | 32.2 (6.5)                            | 29.2 (5.6)           | 33.7 (5.7)                        | 33.6 (5.2)           |
| Mean (SD) gestational age in weeks                            | 19.9 (7.7)           | 21.0 (6.0)           | 21.2 (8.0)                            | 22.6 (6.1)           | 18.3 (7.2)                        | 19.0 (5.5)           |
| Antidepressant use (%)  | 7.1                  | 22.7                 | 14.3                                  | 26.7                 | –                                 | 11.1                 |
| Marital status (%)  |                      |                      |                                       |                      |                                   |                      |
| - Married   | 57.7                 | 65.2                 | 46.7                                  | 69.2                 | 72.7                              | 60.0                 |
| - De Facto  | 34.6                 | 21.7                 | 46.7                                  | 15.4                 | 18.2                              | 30.0                 |
| - Separated   | –                    | 8.7                  | –                                     | 7.7                  | –                                 | 10.0                 |
| - Single  | 7.7                  | 4.3                  | 6.7                                   | 7.7                  | 9.1                               | –                    |
| Birth location (%)  |                      |                      |                                       |                      |                                   |                      |
| - Australia   | 73.1                 | 82.6                 | 66.7                                  | 84.6                 | 81.8                              | 80.0                 |
| - Other   | 26.9                 | 17.4                 | 33.3                                  | 15.4                 | 18.2                              | 20.0                 |
| Income (%)  |                      |                      |                                       |                      |                                   |                      |
| - Up to \$ 20,000   | –                    | 4.5                  | –                                     | –                    | –                                 | 10.0                 |
| - \$ 20,001–\$ 40,000   | 8.0                  | 22.7                 | 7.1                                   | 25.0                 | 9.1                               | 20.0                 |
| - \$ 40,001–\$ 60,000   | 20.0                 | 13.6                 | 28.6                                  | 16.7                 | 9.1                               | 10.0                 |
| - \$ 60,001–\$ 80,000   | 28.0                 | 27.3                 | 21.4                                  | 33.3                 | 36.4                              | 20.0                 |
| - > \$ 80,001   | 32.0                 | 31.8                 | 28.6                                  | 25.0                 | 36.4                              | 40.0                 |
| - Do not wish to divulge                                      | 12.0                 | –                    | 14.3                                  | –                    | 9.1                               | –                    |
| Highest level of education (%)                                |                      |                      |                                       |                      |                                   |                      |
| - Did not finish school                                       | 3.8                  | 12.0                 | 6.7                                   | 21.4                 | –                                 | –                    |
| - High School   | 7.7                  | 24.0                 | 13.3                                  | 21.4                 | –                                 | 27.3                 |
| - Certificate Level/Apprenticeship                            | 23.1                 | 4.0                  | 33.3                                  | –                    | 9.1                               | 9.1                  |
| - Advanced Diploma  | 19.2                 | 4.0                  | 6.7                                   | 7.1                  | 36.4                              | –                    |
| - Bachelor degree   | 11.5                 | 24.0                 | 20.0                                  | 28.6                 | –                                 | 18.2                 |
| - Graduate diploma/certificate                                | 19.2                 | 16.0                 | 6.7                                   | 7.1                  | 36.4                              | 27.3                 |
| - Postgraduate Degree   | 15.4                 | 16.0                 | 13.3                                  | 14.3                 | 18.2                              | 18.2                 |

significance level  $p < 0.01$ . Trends toward lower DNA methylation in the CBT group compared to the TAU group were seen in the *OXTR*, *MEST*, *MEG3*, *H19*, and *CRHR2* genes. Table 6 of the Appendix shows the probes of the candidate genes that were differentially methylated at a nominal significance level  $p < 0.01$ .

#### The glucocorticoid receptor (*NR3C1*) gene and allocation

Mean DNA methylation of 34 promoter-associated *NR3C1* probes (Table 7 in Appendix) did not differ significantly between the intervention and control group (mean  $\Delta\beta = 0.002$ , 95% CI – 0.010 to 0.011). One probe, cg26464411, showed a trend toward lower methylation in the intervention group (Table 7 in Appendix, Fig. 2).

#### Association between genome-wide DNA methylation and baseline depression/anxiety

##### Depression

Linear regression analysis (adjusted for birth weight, HM850 array chip position, sex, age, and allocation) revealed a total of 3065 differentially methylated probes at a nominal significance level ( $p < 0.01$ ) between the groups of children from the antenatally severely depressed women versus the group of children from the antenatally mildly depressed women. Mean DNA methylation values were not significantly different between children born to the severely depressed and the mildly depressed women (mean  $\Delta\beta = 0.0008$  95% CI – 0.007 to 0.008,  $p = 0.95$ ). The top 100 differentially methylated probes according to depression severity at baseline are

**Table 2** Current characteristics of women and their children participating in a DNA methylation study

| Current demographics                  | CBT ( <i>n</i> = 12) | TAU ( <i>n</i> = 11) |
|---------------------------------------|----------------------|----------------------|
| Mean (SD) BDI score                   | 16.1 (13.3)          | 14.9 (11.2)          |
| Mean (SD) BAI score                   | 11.3 (8.9)           | 10.9 (10.2)          |
| Mean (SD) maternal age in years       | 40.0 (4.9)           | 40.6 (4.7)           |
| Antidepressant use, <i>n</i> (%)      | 2 (16.7)             | 6 (54.4)             |
| Mean (SD) child age in years          | 5.7 (1.2)            | 5.9 (1.0)            |
| Mean (SD) child birth weight in grams | 3547 (332)           | 3520 (590)           |
| Gender (boys) (%)                     | 58.3                 | 63.6                 |
| Birth location (%)                    |                      |                      |
| - Australia                           | 81.8                 | 80.0                 |
| - Other                               | 18.2                 | 20.0                 |
| Marital status (%)                    |                      |                      |
| - Married                             | 66.7                 | 54.4                 |
| - De Facto                            | 8.3                  | 18.2                 |
| - Separated                           | 8.3                  | 18.2                 |
| - Single                              | 16.7                 | 9.1                  |
| Highest level of education (%)        |                      |                      |
| - Did not finish school               | –                    | –                    |
| - High School                         | –                    | 27.3                 |
| - Certificate Level/Apprenticeship    | 8.3                  | 9.1                  |
| - Advanced Diploma                    | 8.3                  | –                    |
| - Bachelor degree                     | 25.0                 | 9.1                  |
| - Graduate diploma/certificate        | 41.7                 | 18.2                 |
| - Postgraduate Degree                 | 16.7                 | 36.4                 |
| Income (%)                            |                      |                      |
| - Up to \$ 20,000                     | –                    | 18.2                 |
| - \$ 20,001–\$ 40,000                 | 8.3                  | 18.2                 |
| - \$ 40,001–\$ 60,000                 | –                    | 9.1                  |
| - \$ 60,001–\$ 80,000                 | 8.3                  | 9.1                  |
| - > \$ 80,001                         | 83.3                 | 45.5                 |
| - Do not wish to divulge              | –                    | –                    |
| Smoking <sup>a</sup> (%)              | 8.3                  | 9.1                  |
| Alcohol <sup>b</sup> (%)              | 58.3                 | 27.3                 |

CBT cognitive behavioral therapy, TAU treatment as usual

<sup>a,b</sup>Defined as “currently consuming one or more alcoholic units per week or smoking one or more cigarettes per week”

presented in Table 8 (Appendix). After correcting for multiple testing (corrected  $p \leq 0.01$ ), no probes remained significantly associated with maternal depression severity in pregnancy, prior to treatment.

### Anxiety

Linear regression analysis (adjusted for birth weight, HM850 array chip position, sex, age, and allocation) revealed a total of 3292 differentially methylated probes at a nominal significance level ( $p < 0.01$ ) between the groups of children from

the antenatally severely anxious women versus the group of children from the antenatally mildly anxious women. Mean DNA methylation values were not significantly different between the children born to severely anxious and the mildly anxious women (mean  $\Delta\beta = 0.0002$  95% CI  $-0.004$  to  $0.005$ ,  $p < 0.01$ ). The top 100 differentially methylated probes according to anxiety severity at baseline are presented in Table 9 in Appendix. After correcting for multiple testing (corrected  $p \leq 0.01$ ), no probes remained significantly associated with maternal anxiety severity in pregnancy, prior to treatment.

### Candidate gene-specific DNA methylation and baseline depression/anxiety

#### Depression

Table 10 (Appendix) shows the results of the unpaired Mann-Whitney-Wilcoxon tests, comparing mean DNA methylation of 16 candidate genes between the groups of children from the highly depressed and the mildly depressed women. No genes were significantly differentially methylated at a nominal significance level  $p < 0.01$ . Table 11 of the Appendix shows the probes of the candidate genes that were differentially methylated according to depression symptom severity at a nominal significance level  $p < 0.01$ .

#### Anxiety

Table 12 (Appendix) shows the results of the unpaired Mann-Whitney-Wilcoxon tests, comparing mean DNA methylation of 16 candidate genes between the groups of children from the highly anxious and the mildly anxious women. No genes were significantly differentially methylated at a nominal significance level  $p < 0.01$ . A trend toward higher DNA methylation was seen in the children from the highly anxious mothers compared to the children of mildly anxious mothers in the *MEST* gene. Table 11 of the Appendix shows the probes of the candidate genes that were differentially methylated according to anxiety symptom severity at a nominal significance level  $p < 0.01$ .

### The glucocorticoid receptor (NR3C1) gene and baseline depression/anxiety

#### Depression

Mean DNA methylation of 34 promoter-associated *NR3C1* probes (Table 13, Appendix) did not differ significantly between the groups of children from the highly depressed and the mildly depressed women (mean  $\Delta\beta = 0.006$ , 95% CI  $-0.005$  to  $0.020$ ).

#### Anxiety

Mean DNA methylation of 34 promoter-associated *NR3C1* probes did not differ significantly between the groups of children from the highly anxious and the mildly anxious women (mean  $\Delta\beta = 0.006$ , 95% CI  $-0.005$  to  $0.020$ ). Two



**Table 3** Top 100 differentially methylated probes according to intervention

| CpG        | <i>p</i>    | Adjusted <i>p</i> <sup>a</sup> | Gene              | Gene region | $\Delta\beta$ |
|------------|-------------|--------------------------------|-------------------|-------------|---------------|
| cg19908420 | 3.40E-06    | 0.999997557                    |                   |             | 0.049137862   |
| cg15495292 | 4.01E-06    | 0.999997557                    | <i>AIG1</i>       | Body        | 0.079710136   |
| cg05155812 | 1.56E-05    | 0.999997557                    | <i>SUN1</i>       | TSS1500     | – 0.280713404 |
| cg18818484 | 2.20E-05    | 0.999997557                    | <i>PTCHD2</i>     |             | 0.022078691   |
| cg17622532 | 2.21E-05    | 0.999997557                    |                   |             | 0.024836631   |
| cg14034519 | 2.27E-05    | 0.999997557                    | <i>SNX1</i>       | Body        | 0.053471841   |
| cg26436424 | 3.24E-05    | 0.999997557                    | <i>NGEF</i>       | Body        | 0.033261363   |
| cg21494953 | 3.48E-05    | 0.999997557                    | <i>C5orf23</i>    | TSS1500     | 0.036133838   |
| cg19232929 | 3.58E-05    | 0.999997557                    |                   |             | 0.054387673   |
| cg22342380 | 3.86E-05    | 0.999997557                    |                   |             | 0.03688025    |
| cg13719771 | 5.98E-05    | 0.999997557                    | <i>NDUFA9</i>     | Body        | 0.13765872    |
| cg10356363 | 6.06E-05    | 0.999997557                    | <i>CEBPB</i>      | TSS1500     | 0.026639222   |
| cg05205351 | 6.20E-05    | 0.999997557                    | <i>NOP56</i>      | Body        | 0.05930508    |
| cg14231326 | 6.23E-05    | 0.999997557                    |                   |             | 0.031289864   |
| cg14358699 | 7.14E-05    | 0.999997557                    |                   |             | 0.047991502   |
| cg06961812 | 8.01E-05    | 0.999997557                    | <i>PRODH2</i>     | Body        | 0.058582642   |
| cg16007230 | 8.39E-05    | 0.999997557                    | <i>ABCC3</i>      | ExonBnd     | 0.036161879   |
| cg25968469 | 8.53E-05    | 0.999997557                    | <i>ARHGAP22</i>   | Body        | 0.056699144   |
| cg23619591 | 8.80E-05    | 0.999997557                    | <i>C19orf81</i>   | Body        | 0.057592082   |
| cg09240747 | 0.000101189 | 0.999997557                    |                   |             | 0.067301777   |
| cg18077049 | 0.000101567 | 0.999997557                    | <i>GLRA3</i>      | Body        | 0.116790545   |
| cg24435401 | 0.000110721 | 0.999997557                    | <i>NPAS4</i>      | TSS1500     | 0.021387283   |
| cg23274420 | 0.000110944 | 0.999997557                    |                   |             | 0.068615769   |
| cg09223928 | 0.000111509 | 0.999997557                    |                   |             | 0.030359585   |
| cg18666104 | 0.000115314 | 0.999997557                    | <i>CORO1C</i>     | Body        | 0.058415174   |
| cg16273469 | 0.000115391 | 0.999997557                    |                   |             | 0.036049214   |
| cg00541777 | 0.000120288 | 0.999997557                    | <i>COLEC11</i>    | TSS1500     | 0.120518141   |
| cg06646082 | 0.0001208   | 0.999997557                    | <i>BTBD17</i>     | TSS1500     | 0.0430183     |
| cg03711840 | 0.000127893 | 0.999997557                    | <i>PLXNA1</i>     | Body        | 0.043191584   |
| cg19465002 | 0.000130791 | 0.999997557                    |                   |             | 0.033852961   |
| cg14687471 | 0.000134464 | 0.999997557                    | <i>NBR2</i>       | Body        | 0.023128809   |
| cg27243560 | 0.000134814 | 0.999997557                    |                   |             | 0.031689225   |
| cg05510714 | 0.000135017 | 0.999997557                    | <i>KYNU</i>       | Body        | 0.153887531   |
| cg12987887 | 0.000136898 | 0.999997557                    | <i>UPB1</i>       | ExonBnd     | – 0.01972518  |
| cg26836955 | 0.000138572 | 0.999997557                    | <i>LONP1</i>      | Body        | 0.039104166   |
| cg26330841 | 0.000138665 | 0.999997557                    |                   |             | 0.032962344   |
| cg16720807 | 0.000142967 | 0.999997557                    | <i>FAM176A</i>    | 5'UTR       | 0.042119403   |
| cg01440210 | 0.000143289 | 0.999997557                    |                   |             | 0.030341728   |
| cg17068417 | 0.000144326 | 0.999997557                    | <i>EEFSEC</i>     | Body        | 0.030665165   |
| cg15313810 | 0.000144443 | 0.999997557                    | <i>ST6GALNAC4</i> | Body        | 0.029787439   |
| cg07545731 | 0.000147518 | 0.999997557                    | <i>COL22A1</i>    | Body        | 0.04468122    |
| cg14684297 | 0.000150469 | 0.999997557                    | <i>ARHGAP33</i>   | 5'UTR       | 0.032019831   |
| cg10727673 | 0.000154265 | 0.999997557                    | <i>TMEM22</i>     | TSS1500     | 0.089444195   |
| cg04798314 | 0.000155738 | 0.999997557                    | <i>SMYD3</i>      | Body        | 0.323390033   |

**Table 3** Top 100 differentially methylated probes according to intervention (*Continued*)

| CpG        | <i>p</i>    | Adjusted <i>p</i> <sup>a</sup> | Gene            | Gene region | Δβ            |
|------------|-------------|--------------------------------|-----------------|-------------|---------------|
| cg11035122 | 0.000160944 | 0.999997557                    | <i>MIR758</i>   | TSS1500     | 0.055539324   |
| cg12360330 | 0.000168181 | 0.999997557                    | <i>CENPJ</i>    | Body        | 0.032193572   |
| cg07469546 | 0.000172234 | 0.999997557                    |                 |             | 0.014405304   |
| cg17785398 | 0.000172977 | 0.999997557                    | <i>KCNJ6</i>    | Body        | 0.022656857   |
| cg18291664 | 0.000173083 | 0.999997557                    | <i>PRKAR1B</i>  | Body        | 0.040654976   |
| cg09319487 | 0.000181803 | 0.999997557                    |                 |             | 0.033053753   |
| cg11510586 | 0.000186082 | 0.999997557                    |                 |             | 0.107251714   |
| cg25441526 | 0.000188457 | 0.999997557                    | <i>WDFY4</i>    | Body        | 0.025251026   |
| cg19379103 | 0.000188787 | 0.999997557                    | <i>SSBP3</i>    | Body        | 0.031870653   |
| cg19769811 | 0.00019183  | 0.999997557                    | <i>RASGRF2</i>  | TSS1500     | 0.046395706   |
| cg26221509 | 0.000199233 | 0.999997557                    | <i>SCUBE1</i>   | Body        | 0.039685931   |
| cg14700416 | 0.000199451 | 0.999997557                    | <i>SPOCK3</i>   | 5'UTR       | 0.049430209   |
| cg22746421 | 0.000200331 | 0.999997557                    |                 |             | 0.02669027    |
| cg23553242 | 0.000200938 | 0.999997557                    | <i>USP2</i>     | Body        | 0.043740484   |
| cg06617093 | 0.000206244 | 0.999997557                    |                 |             | 0.032231234   |
| cg08670534 | 0.000206305 | 0.999997557                    | <i>COL2A1</i>   | Body        | 0.032117847   |
| cg15791944 | 0.000212127 | 0.999997557                    |                 |             | 0.055152706   |
| cg17562896 | 0.000216404 | 0.999997557                    | <i>SV2C</i>     | Body        | 0.037479302   |
| cg02018176 | 0.000217297 | 0.999997557                    | <i>KIAA1530</i> | Body        | 0.047057842   |
| cg11576176 | 0.000220243 | 0.999997557                    | <i>GSX2</i>     | 1stExon     | 0.03556139    |
| cg09480336 | 0.0002295   | 0.999997557                    | <i>POLD1</i>    | Body        | 0.03212232    |
| cg21592262 | 0.000233681 | 0.999997557                    |                 |             | 0.06371313    |
| cg12472342 | 0.000234117 | 0.999997557                    |                 |             | − 0.069235248 |
| cg18361948 | 0.00023564  | 0.999997557                    |                 |             | 0.029932491   |
| cg00945089 | 0.000236572 | 0.999997557                    | <i>GFRA1</i>    | Body        | 0.033266209   |
| cg07442357 | 0.000238546 | 0.999997557                    |                 |             | 0.01892614    |
| cg09193498 | 0.000239232 | 0.999997557                    | <i>SEZ6</i>     | Body        | 0.042776024   |
| cg02438610 | 0.000240811 | 0.999997557                    | <i>SUN1</i>     | TSS1500     | − 0.013139753 |
| cg15037661 | 0.00024103  | 0.999997557                    | <i>NR1D2</i>    | TSS1500     | 0.00946764    |
| cg26264656 | 0.000243011 | 0.999997557                    | <i>SKI</i>      | Body        | 0.034797294   |
| cg24367840 | 0.000243465 | 0.999997557                    | <i>PSMD14</i>   | Body        | 0.057487682   |
| cg05289897 | 0.000259274 | 0.999997557                    |                 |             | 0.012403078   |
| cg16419764 | 0.000261486 | 0.999997557                    | <i>CDYL</i>     | Body        | 0.026043028   |
| cg00248302 | 0.000266776 | 0.999997557                    | <i>FCRL5</i>    | Body        | 0.028022889   |
| cg24900542 | 0.000269678 | 0.999997557                    |                 |             | 0.085875055   |
| cg15078841 | 0.000272298 | 0.999997557                    |                 |             | 0.022837528   |
| cg12541879 | 0.000282436 | 0.999997557                    | <i>PTPRN2</i>   | Body        | 0.056208383   |
| cg01976641 | 0.000283246 | 0.999997557                    |                 |             | 0.05497368    |
| cg17121322 | 0.000286514 | 0.999997557                    |                 |             | 0.025193249   |
| cg17547875 | 0.000288231 | 0.999997557                    |                 |             | 0.01236688    |
| cg18169610 | 0.000296554 | 0.999997557                    | <i>CD81</i>     | Body        | 0.038708      |
| cg04801704 | 0.000304651 | 0.999997557                    | <i>TLL2</i>     | Body        | 0.025096532   |
| cg23425290 | 0.000307508 | 0.999997557                    | <i>ABCC1</i>    | Body        | 0.023343856   |
| cg22680931 | 0.00030882  | 0.999997557                    | <i>TMEM167B</i> | TSS1500     | 0.122894387   |



**Table 3** Top 100 differentially methylated probes according to intervention (Continued)

| CpG        | <i>p</i>    | Adjusted <i>p</i> <sup>a</sup> | Gene             | Gene region | Δβ          |
|------------|-------------|--------------------------------|------------------|-------------|-------------|
| cg01723825 | 0.000310423 | 0.999997557                    | <i>URI1</i>      | TSS200      | 0.039217642 |
| cg16261251 | 0.000311941 | 0.999997557                    |                  |             | 0.06722457  |
| cg01400541 | 0.000314878 | 0.999997557                    | <i>C10orf128</i> | Body        | 0.042719378 |
| cg26796807 | 0.000318004 | 0.999997557                    |                  |             | 0.04717045  |
| cg10038145 | 0.000319876 | 0.999997557                    | <i>POR</i>       | Body        | 0.045738894 |
| cg09078103 | 0.000320468 | 0.999997557                    | <i>SNX9</i>      | Body        | 0.027261168 |
| cg08880699 | 0.000322485 | 0.999997557                    |                  |             | 0.043133838 |
| cg03116452 | 0.00032398  | 0.999997557                    | <i>PLD3</i>      | 5'UTR       | 0.034421382 |
| cg03071994 | 0.000324145 | 0.999997557                    | <i>NR4A1</i>     | Body        | 0.029626215 |
| cg21485062 | 0.000324634 | 0.999997557                    | <i>C7orf25</i>   | Body        | 0.024308813 |
| cg11504793 | 0.000326763 | 0.999997557                    | <i>NOL4L</i>     | Body        | 0.025196146 |
| cg04837576 | 0.00032871  | 0.999997557                    | <i>ADRBK2</i>    | Body        | 0.030823149 |

Δβ = mean β (treatment as usual) – mean β (cognitive behavioral therapy)

TSS transcription start site, UTR untranslated region

<sup>a</sup>Adjusted for multiple testing [45]

probes, cg07515400 and cg22402730, showed a trend toward higher DNA methylation in the children from severely anxious mothers (Table 13, Appendix).

## Discussion

In this follow-up of one of the first randomized controlled trials on the effect of antenatal psychological depression treatment (CBT) on children's DNA methylation patterns, we found no robust evidence of widespread methylation differences between children of women in the control or intervention group. However, at a pre-specified nominal significance level of  $p < 0.01$ , 4780 differentially methylated probes according to allocation pointed to an overall 2.7% lower DNA methylation level of probes in children from the intervention group. Applying a candidate approach, non-significant trends toward lower DNA methylation in the intervention group were seen in *OXTR*, *MEST*, *MEG3*, *H19*, and *CRHR2*. We did not find a significant difference in

mean DNA methylation of 34 *NR3C1* promoter-associated probes between the intervention and control groups. Nevertheless, the majority of probes (68%) showed lower DNA methylation in the intervention group compared to the control group, with cg26464411 as topmost differentially methylated probe, a CpG site that has been associated with depression in earlier studies [25, 26]. Whether these trends are persistent and clinically relevant remains to be determined in future studies with larger sample size and longer follow-up.

Of the top five probes that were most differentially methylated between the intervention and the control group, three corresponded to annotated genes: cg15495292 on the *AIG1* gene, which is a gene involved in androgen regulation; cg18818484 on the *PTCHD2* gene, which is involved in neuronal proliferation and differentiation; and cg05155812 on *SUN1*, a gene that potentially plays a role in neuronal migration and cerebellar development. These findings may be

**Table 4** Top 10 differentially methylated genes according to allocation

| CpG        | <i>p</i> | Adjusted <i>p</i> <sup>a</sup> | Gene           | Gene region | Δβ           |
|------------|----------|--------------------------------|----------------|-------------|--------------|
| cg19908420 | 3.40E-06 | 0.999998                       |                |             | 0.049137862  |
| cg15495292 | 4.01E-06 | 0.999998                       | <i>AIG1</i>    | Body        | 0.079710136  |
| cg05155812 | 1.56E-05 | 0.999998                       | <i>SUN1</i>    | TSS1500     | -0.280713404 |
| cg18818484 | 2.20E-05 | 0.999998                       | <i>PTCHD2</i>  | Body        | 0.022078691  |
| cg17622532 | 2.21E-05 | 0.999998                       |                |             | 0.024836631  |
| cg14034519 | 2.27E-05 | 0.999998                       | <i>SNX1</i>    | Body        | 0.053471841  |
| cg26436424 | 3.24E-05 | 0.999998                       | <i>NGEF</i>    | Body        | 0.033261363  |
| cg21494953 | 3.48E-05 | 0.999998                       | <i>CSorf23</i> | TSS1500     | 0.036133838  |
| cg19232929 | 3.58E-05 | 0.999998                       |                |             | 0.054387673  |
| cg22342380 | 3.86E-05 | 0.999998                       |                |             | 0.03688025   |

Δβ = mean β(TAU) – mean β(CBT)

CBT cognitive behavioral therapy, TAU treatment as usual, TSS transcription start site, UTR untranslated region

<sup>a</sup>Corrected for multiple testing [46]

relevant as the desired effect of a prenatal intervention would be to target genes that mediate the associations of prenatal stress, depression or anxiety with adverse neurodevelopmental disorders in children [27, 28]. Our results are promising, but evidently replication in larger studies is necessary.

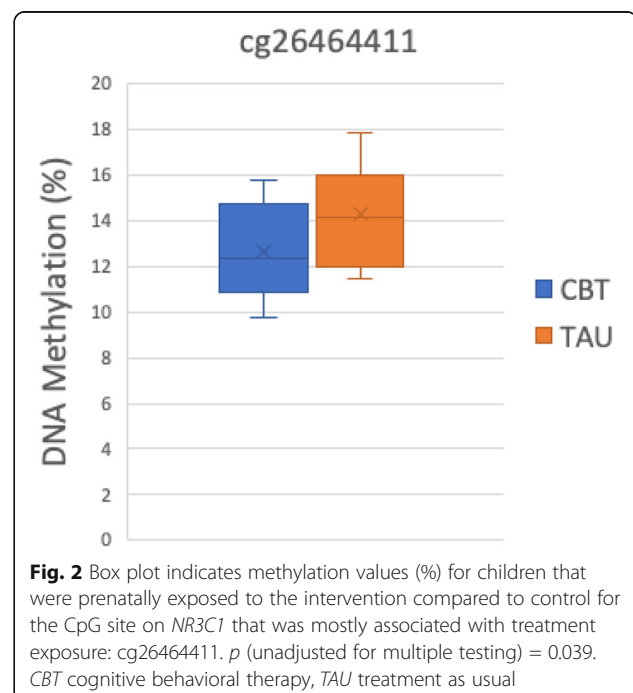
Additionally, we revealed trends toward lower DNA methylation in children from the intervention group compared to the control group in 5 out of 16 candidate genes that have previously been associated with prenatal exposure to maternal stress, depression, or anxiety. These trends were observed in *OXTR*, the gene coding for the Oxytocin receptor; the *MEST* gene, a gene involved in metabolism; *MEG3*, a long noncoding RNA; *H19*, an imprinted gene; and *CRHR1*, a gene for corticotrophin releasing hormone receptors. We did not find a significant difference in mean DNA methylation between the intervention and control group on the promoter region of the *NR3C1* gene, coding for the glucocorticoid receptor. Nevertheless, cg26464411 showed a trend toward lower DNA methylation in the intervention group. This CpG site has been positively correlated with depressive symptoms or hypercortisolism in earlier studies [25, 26]. Although our results were not significant, the trends we have observed were in line with our expectations, based on earlier findings from observational studies showing increased methylation of *NR3C1* in newborns and young children of antenatally stressed, depressed, or anxious women [20, 29], which was associated with increased stress responses [21, 30].

The women in the current study were treated at a mean of 18.6 weeks gestational age, and it may be possible that the effect of treatment on offspring DNA methylation would have been stronger if the women had been treated earlier in their pregnancies. Increased attention is currently focused on the period of early pregnancy, and even the preconception period, as an important time window for adverse environmental factors inducing prenatal programming, which has been shown in animal studies [18]. Further evidence in humans is derived from studies examining prenatal famine, in which the largest effect on offspring methylation was found after prenatal exposure to undernutrition in early pregnancy [31]. We did not test for an interaction between allocation status and gestational age on mean methylation in candidate genes because of the lack of significance in the initial analyses, but in larger future studies, exploring moderation through gestational age would be highly informative to identify treatment effects on DNA methylation during specific stages of pregnancy.

A limitation of the study was a lack of statistical power, as we were only able to include approximately half (23/54 = 43%) of the original sample in this follow-up. Nevertheless, associations between prenatal stress and methylation status of *NR3C1* have been reported in studies with a similar sample size [30, 32]. It was of interest that women who

participated in the current follow-up study had lower levels of depression and anxiety at baseline compared to the participants that were lost to follow-up (Table 1). Also, they were observed to have higher incomes and were more highly educated at baseline. However, attrition bias is not likely to have occurred as this was the case in both groups [33]. Despite no formal statistical tests being conducted [34], it was evident that the difference in anxiety (BAI) scores before and after treatment between the intervention and control group was twice as high in the non-responders compared to the responders (14.5 versus 7.5), indicating that women with greater response to treatment were relatively underrepresented in the current sample. Additionally, some women in the control group also reported accessing psychological or medical treatment outside the trial [24]. This, and the lower participation of those who responded better to treatment, might have led to an underestimation of the effect of therapy on methylation profiles in the children in the current study.

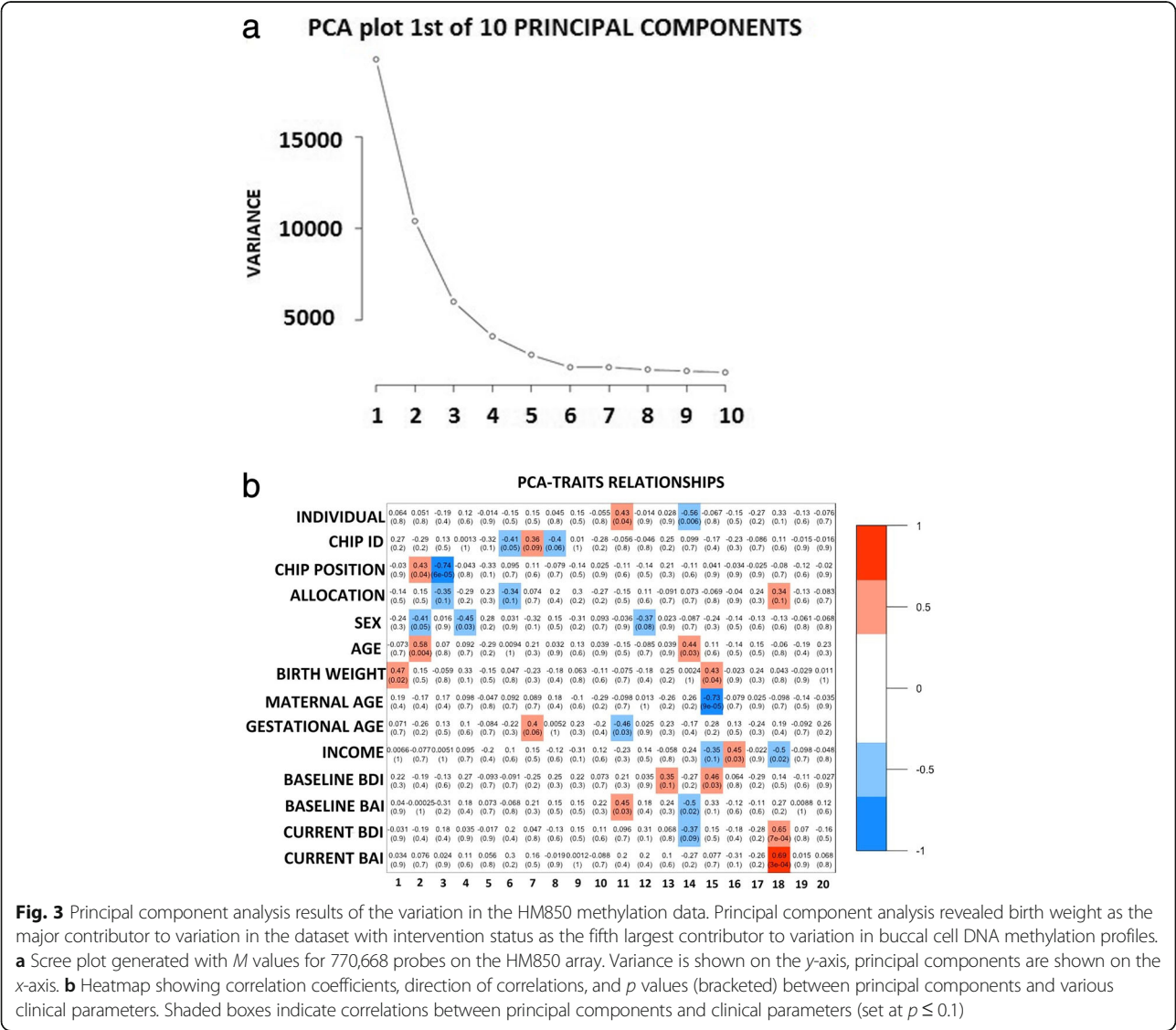
Although both groups were reasonably balanced in terms of psychological and sociodemographic factors at the time of follow-up, it is still possible that other, unmeasured factors are (partly) responsible for the trends observed in the children's epigenetic profiles according to allocation status. Because of the small sample size of our study, we chose to include only those variables that were likely to attribute mostly to the variation in DNA methylation, such as child gender, age, birth weight, and income. We did not include educational attainment, although this also appeared to be somewhat higher in the intervention group (although not statistically significant, results not shown). In addition,



maternal body composition in pregnancy, pregnancy complications, and mode of delivery were not recorded in the original study files, and hence, not included in the current study. As these factors may act as mediators in the causal path from improved mood in pregnancy to better child outcomes, in future studies these variables should be included as well. Nevertheless, we did have access to the children's birth weight, an important marker for general health of the baby, which showed to be similar between both groups. Also, we were unable to control for PC5 in the analyses, as none of the variables included in the model was associated with PC5. Nevertheless, the contribution fraction of PC5 to the variation in DNA methylation was very marginal compared to the contribution fraction of PC1, PC2, PC3, and PC4, which were associated with known variables and therefore were controlled for in our analyses. Finally, we did not adjust for cellular heterogeneity in our study. The most

widely applied method is the reference-based deconvolution method originally described by Houseman et al., which permits the estimation of the proportion of various cell types within a sample based on existing reference data sets [35]. For blood, several studies have analyzed the methylation profile of the specific cell- types present in whole blood, which can serve as reference data. However, for saliva, this has not been performed systematically, but studies that have applied the Houseman deconvolution method on salivary genome wide DNA methylation data (combining reference methylomes from leucocyte subtypes and buccal epithelial cells references methylomes) have shown that saliva is less heterogenic compared to blood [36].

The impact of the postnatal environment on methylation profiles in children also cannot be ignored. Exposure to stressful life events from birth to adolescence has been associated with higher *NR3C1* methylation [37]. Although in



**Fig. 3** Principal component analysis results of the variation in the HM850 methylation data. Principal component analysis revealed birth weight as the major contributor to variation in the dataset with intervention status as the fifth largest contributor to variation in buccal cell DNA methylation profiles. **a** Scree plot generated with *M* values for 770,668 probes on the HM850 array. Variance is shown on the y-axis, principal components are shown on the x-axis. **b** Heatmap showing correlation coefficients, direction of correlations, and *p* values (bracketed) between principal components and various clinical parameters. Shaded boxes indicate correlations between principal components and clinical parameters (set at  $p \leq 0.1$ )

both intervention and control group, more women were currently using antidepressant medication compared to when they were pregnant at enrollment of the original study, this was much more pronounced in the control group (relative increase of 43.3%) compared to the intervention group (relative increase of 16.7%). These observations may be consistent with a potential longer-term beneficial effect of treatment in the women, which in turn, might have positively affected child outcomes. Women from the intervention group also reported higher incomes compared to baseline, which was not the case in the control group, although including income as additional covariate did not significantly alter the results. To be able to isolate the effect of antenatal CBT on offspring DNA methylation in utero, prior to any postnatal confounding, evidence from trials that include cord blood and/or placenta samples for DNA methylation (and gene expression) are needed.

Finally, it has not yet been fully elucidated how maternal depression affects child adversity. Nevertheless, epigenetic modification of fetal genes in response to increased cortisol exposure, either directly or via a decrease in placental inactivation, has been widely accepted as a potential underlying mechanism. Although our study findings could not robustly support this hypothesis, the trends observed are in line with earlier evidence. The existing evidence is nearly exclusively based on findings from experiments in animals and observational human studies. The fact that the exploratory findings from this novel experimental study in humans are in line with the available evidence is therefore promising. It must be noted that we mostly looked at statistically significant results at an uncorrected  $p$ -value level. The results of our study should therefore be interpreted with caution. Although the observed effect sizes were small, with mean differences of 1–5% in methylation status, they are in line with earlier evidence [20]. Because of the lack of studies with a comparable study design, it is not yet possible to replicate our findings in a similar trial; however, plans for a larger trial are currently in progress.

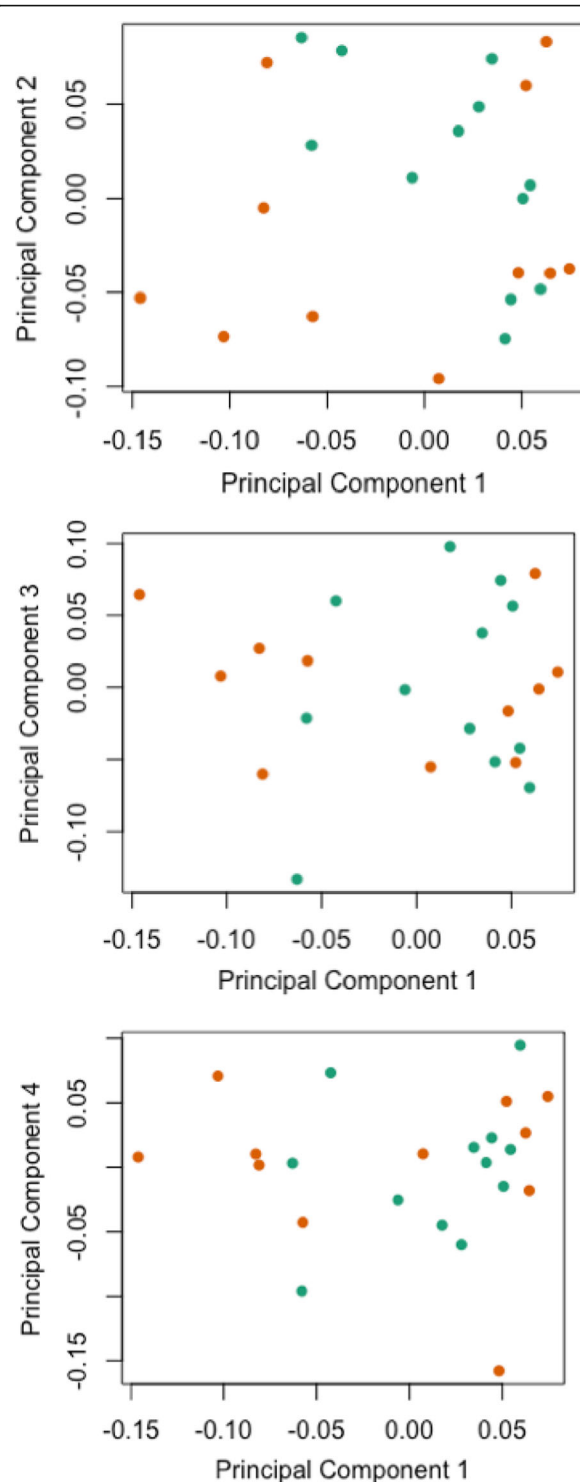
## Conclusion

We found preliminary evidence of a possible effect of cognitive behavioral therapy during pregnancy on widespread methylation and a non-significant trend towards lower methylation of a specific CpG site previously linked to depressive symptoms and child maltreatment in the intervention group. However, none of the effects survived correction for multiple testing. Larger studies are now warranted.

## Methods

### Study population

For the BBB study, women aged 18 years or over, and less than 30 weeks pregnant were recruited through screening programs at the Northern Hospital and Mercy Hospital for Women, Melbourne, Australia, and via



**Fig. 4** MDS plots, with samples colored according to CBT (turquoise)/TAU (orange) status, showing the relatedness of samples over the first four principal components of variation. CBT cognitive behavioral therapy, TAU treatment as usual



other health professionals and services in the public (e.g., obstetricians, GPs, and PaNDA; a Perinatal Anxiety and Depression helpline) and private sector (e.g., Northpark Private Hospital). The participating institutions were reached through advertisement and encouraged to refer women with suspected clinical depression. Women scoring 13 points or higher on the Edinburgh Postnatal Depression Scale (EPDS), the optimal score for detecting depression during pregnancy [38], were referred to the study for assessment by a psychologist if they consented. They were included in the study if they met DSM-IV criteria for a minor or major depressive disorder or an adjustment disorder with mixed depression and anxiety [39]. Severity of depression and anxiety symptoms was measured with the Beck Depression and Anxiety Inventories [40, 41]. Women with comorbid axis I disorders or medical conditions that were likely to interfere with study participation, risk requiring crisis management, participation in other psychological programs, or significant difficulty with English were excluded [24]. Women included in the study ( $N=54$ ) were randomized to receive pregnancy-specific CBT ( $N=28$ ) or TAU ( $N=26$ ). The CBT program consisted of seven individual sessions and one partner-session. TAU consisted of case-management by a midwife or a general practitioner and referral to other services of agencies as necessary. For ease of interpretation, in the results sections of this paper, the group of children of mothers from the CBT group will be referred to as the “intervention” group, and the group of children of mothers from the TAU group will be referred to as the “control” group. For participation in the current study, starting approximately 5 years after the BBB program had ended, all participants were invited through a letter. If they agreed to participate, an appointment at the Melbourne Brain Institute was planned, and informed consent was signed prior to or on the day of their visit to the clinic. If women were not able to attend the clinic, they were invited to send a buccal sample through the mail. The study was approved by the Human Research Ethics Committees of Austin Health, Melbourne, Australia.

#### Data collection

A questionnaire on current sociodemographic data and current symptoms of depression and anxiety was sent to each woman's home address. Baseline demographics, including symptoms of depression and anxiety as well as the child's birth weight, were taken from the BBB study files. At the Melbourne Brain Centre, a cognitive assessment by means of the Wechsler Preschool and Primary Intelligence Scale (WWPSI-III) [42] was performed on the child, an MRI scan of the child's brain was conducted, of which results are described elsewhere, and a buccal cell sample from the child was obtained by a researcher who was blinded to the allocation status of the women.

#### Buccal cell samples

Buccal cells were collected using a dedicated swab (OraCollect 100, DNA Genotek Inc., Ontario, Canada). Children were instructed not to eat or drink 30 min prior to taking the swab. Women who were not able to visit the Melbourne Brain Centre were instructed how to apply the swab on their child, and asked to send the sample via mail. The swabs were stored at room temperature at the Parent-Infant Research Institute and transported to the Murdoch Children's Research Institute (Melbourne, Australia) for DNA extraction within 2 weeks after collection.

#### DNA extraction and genome-wide methylation detection

DNA extraction of all samples was performed using the NucleoBond CB20 DNA extraction kit. Purification of DNA was assessed using Nanodrop Spectrophotometry. Bisulfite conversion was performed using the EZ-96 DNA methylation kit (ZYMO Research Corporation) according to the manufacturer's instructions. DNA methylation profiling was performed at the Australian Genome Research Facility, on bisulfite converted DNA using the Illumina Infinium Methylation EPIC BeadChip Array (HM850) (Illumina), which measures CpG methylation at > 850,000 genomic sites.

#### Candidate gene approach

We extracted 729 probes spanning 16 a priori selected genes for linear regression analysis. Candidate genes were those that had previously been assessed in relation to prenatal exposure to maternal stress, depression, and/or anxiety in earlier studies [20]. Genes of interest were genes encoding brain-derived neurotrophic factor (*BDNF*; 91 probes), corticotrophin releasing hormone (*CRH*; 21 probes), corticotrophin-releasing factor-binding protein (*CRHBP*; 25 probes), corticotrophin-releasing hormone receptors 1 and 2 (*CRHR1*; 41 probes, *CRHR2*; 40 probes), FK506 binding protein (*FKBP5*; 49 probes), a long noncoding RNA (*H19*; 57 probes), hydroxysteroid 11-beta dehydrogenase 1 and 2 (*HSD11B1*; 25 probes, *HSD11B2*; 23 probes), insulin-like growth factor (*IGF2*; 15 probes), maternally expressed 3 (*MEG3*; 87 probes), mesoderm-specific transcript homolog protein (*MEST*; 63 probes), the glucocorticoid receptor (*NR3C1*; 89 probes), the mineralocorticoid receptor (*NR3C2*; 50 probes), the oxytocin receptor (*OXT*; 22 probes), and the serotonin transporter (*SLC6A4*; 31 probes) [20]. Additionally, considering the especially strong evidence for this gene, we separately analyzed the probes of the promoter region of the glucocorticoid receptor gene (*NR3C1* promoter-associated probes; 34 probes) for differential methylation.

#### Statistical analysis

DNA methylation was defined as a continuous variable varying from 0 (completely unmethylated) to 1 (completely methylated). Methylation data were processed in R using

the *minfi* package. Normalization of the data was performed using the SWAN method [43]. Probes on X and Y chromosomes, probes that were associated with SNPs with a minor allele frequency > 1%, and cross-reactive probes [44] were removed from the dataset. This resulted in data for 770,668 probes available for subsequent analysis.

#### Sources of variation

Main contributors to the variation in the methylation data were identified by principal component analysis (PCA). We included the following variables in the analysis to assess associations with PC's: participant ID, chip ID, HM850 array chip position, allocation, sex, child age, birth weight, maternal age, gestational age, current income, baseline depression symptoms, baseline anxiety symptoms, current depression symptoms, and current anxiety symptoms. Results of the PCA showed that the first five principal components contributed most to the variation in the methylation data, and all variables associated with any of these PC's were added as covariate in all analyses (Fig. 3a). The heatmap demonstrated that allocation was associated with the third principal component. Birth weight, child age, sex, and HM850 array chip position were associated with the first four principal components and they were included in the analyses as covariates. None of the variables included in our model was significantly associated with the fifth principal component, and this PC was therefore not included in our model as covariate (Fig. 3b). Unsupervised analysis by multidimensional scaling was conducted in order to examine sources of variation within the dataset. Beta values (methylation level) at all HM850 probes for all samples were used to produce multidimensional scaling (MDS) plots, with samples colored according to intervention (turquoise)/control (orange) status, showing the relatedness of samples over the first two principal components of variation (Fig. 4a). Coloring by intervention/control revealed no distinct separation by allocation. Additional MDS plots of samples over other principal components also failed to show a distinct separation between the two groups (Figs. 4b c).

#### Differential methylation according to allocation

Linear regression analysis was used to identify associations between the intervention status and epigenome-wide DNA methylation. We took into account variation associated with the covariates birth weight, HM850 array chip position, child sex and age, to account for PC1, PC2, PC3, and PC4, as identified by PCA. The Benjamini-Hochberg False-Discovery-Rate method [45] was used to correct for multiple testing. However, none of the analyses yielded significant differentially methylated probes between the intervention and control group after correcting for multiple testing.

In an explorative analysis, we extracted differentially methylated probes between the intervention and control group at a nominal significance level set at  $p < 0.01$ , prior to correcting for multiple testing. We assessed differences in mean DNA methylation of all significant probes between the intervention and control group using an unpaired Mann-Whitney-Wilcoxon test. We additionally compared mean beta differences of 16 candidate genes, and the promoter region of the *NR3C1* gene between the intervention and control group using an unpaired Mann-Whitney-Wilcoxon test.

#### Differential methylation according to baseline depression or anxiety symptom score

As additional explorative analyses, two separate linear regression models were also used to investigate associations between baseline depression (BDI-II score) and baseline anxiety (BAI- score) with methylation profiles in the children. For ease of interpretation, the sample was divided into two groups in both analyses. The rationale behind this approach was to explore widespread methylation variation between women with severe symptoms compared to those with mild symptoms using clinically relevant cut-offs, rather than investigating the direction of correlations between increasing depression and anxiety scores on all probes separately. Baseline depression was converted to a dichotomous variable using clinically relevant Beck questionnaire cut-offs. Women with BDI-II  $\geq 29$  were classified as "highly depressed" ( $n = 13$ ), whereas those with a score below 29 were classified as "mildly depressed" ( $n = 9$ ) [46]. This procedure was repeated for baseline anxiety (BAI-score). The cut-off for clinically relevant anxiety is set at 16, and therefore we classified women with BAI  $\geq 16$  as "highly anxious" ( $n = 8$ ), and women with BAI below 16 as "mildly anxious" ( $n = 14$ ) [47]. One woman had missing data on baseline depression and anxiety and was excluded from the analysis. We took into account allocation status, birth weight, HM850 array chip position, child sex, and age as covariates, as identified by PCA. Differentially methylated probes at a nominal significance level set at  $p < 0.01$ , prior to correction for multiple testing, were extracted. We compared differences in mean DNA methylation in groups of children of women with high baseline symptoms and low baseline symptoms using an unpaired Mann-Whitney-Wilcoxon test, both for depression and anxiety. We additionally compared mean beta differences of 16 candidate genes, and the promoter region of the *NR3C1* gene between groups of children of women with high baseline symptoms and low baseline symptoms using an unpaired Mann-Whitney-Wilcoxon test, both for depression and anxiety.



## Appendix

**Table 5** Differential mean methylation of candidate genes in buccal cell DNA of children after maternal antenatal CBT or TAU

| Gene                  | $\Delta\beta$ | 95%CI               | P     |
|-----------------------|---------------|---------------------|-------|
| <i>NR3C1</i>          | 0.004         | −0.004 to 0.011     | 0.32  |
| <i>NR3C1</i> Promoter | 0.002         | −0.010 to 0.011     | 0.65  |
| <i>SLC6A4</i>         | 0.013         | −0.007 to 0.035     | 0.09  |
| <i>OXTR</i>           | 0.008         | −4.7e-05 to 1.6e-02 | 0.04  |
| <i>NR3C2</i>          | 0.002         | −0.005 to 0.009     | 0.6   |
| <i>MEST</i>           | 0.013         | 0.003 to 0.024      | 0.02  |
| <i>MEG3</i>           | 0.012         | 0.00004 to 0.023    | 0.04  |
| <i>IFG2</i>           | 0.005         | −0.014 to 0.028     | 0.65  |
| <i>HSD11B1</i>        | 0.004         | −0.0123 to 0.019    | 0.61  |
| <i>HSD11B2</i>        | 0.003         | −0.003 to 0.010     | 0.29  |
| <i>H19</i>            | 0.019         | 0.003 to 0.041      | 0.03  |
| <i>CRHR1</i>          | 0.013         | −0.0003 to 0.027    | 0.06  |
| <i>CRHR2</i>          | 0.019         | 0.002 to 0.032      | 0.02  |
| <i>CRHRBP</i>         | −0.003        | −0.033 to 0.033     | 0.93  |
| <i>CRH</i>            | 0.001         | −0.014 to 0.015     | 0.98  |
| <i>BDNF</i>           | 0.001         | −0.005 to 0.008     | 0.38  |
| <i>FKBP5</i>          | 0.006         | −0.0003 to 0.0139   | 0.051 |

$\Delta\beta$  = mean  $\beta$  (TAU) - mean  $\beta$  (CBT)

CBT cognitive behavioral therapy, TAU treatment as usual

**Table 6** Probes in candidate gene analysis showing differential methylation according to intervention at uncorrected  $p < 0.01$

| CpG        | $p$         | Adjusted $p^a$ | Gene            | Gene region | $\Delta\beta$ |
|------------|-------------|----------------|-----------------|-------------|---------------|
| cg27338480 | 0.002299634 | 0.999997557    | <i>MEST</i>     | 5'UTR       | 0.036568643   |
| cg25579735 | 0.004343149 | 0.999997557    | <i>NR3C1</i>    | 5'UTR       | −0.028037036  |
| cg01913022 | 0.0064351   | 0.999997557    | <i>CRHR2</i>    | TSS1500     | 0.068307524   |
| cg03366382 | 0.006909299 | 0.999997557    | <i>INS-IGF2</i> | TSS1500     | 0.044997291   |
| cg03128167 | 0.009155461 | 0.999997557    | <i>IGF2</i>     | Body        | 0.017691809   |

$\Delta\beta$  = mean  $\beta$  (treatment as usual) - mean  $\beta$  (cognitive behavioral therapy)

TSS transcription start site, UTR untranslated region

<sup>a</sup>Adjusted for multiple testing [45]

**Table 7** Differential methylation according to intervention (promoter-associated *NR3C1* probes)

| CpG        | $p$         | adjusted $P^1$ | $\Delta\beta$ |
|------------|-------------|----------------|---------------|
| cg26464411 | 0.038765207 | 0.999997557    | 0.016954389   |
| cg07515400 | 0.080810513 | 0.999997557    | −0.006695682  |
| cg10847032 | 0.097881389 | 0.999997557    | 0.002994888   |
| cg06952416 | 0.1418427   | 0.999997557    | 0.022027436   |
| cg06968181 | 0.220252023 | 0.999997557    | 0.007404024   |
| cg18019515 | 0.226633505 | 0.999997557    | 0.002112324   |
| cg04111177 | 0.239037451 | 0.999997557    | −0.002860936  |
| cg18068240 | 0.254658402 | 0.999997557    | 0.002064659   |
| cg21209684 | 0.270282959 | 0.999997557    | 0.002460768   |

**Table 7** Differential methylation according to intervention (promoter-associated *NR3C1* probes) (Continued)

| CpG        | <i>p</i>    | adjusted <i>P</i> <sup>a</sup> | Δβ            |
|------------|-------------|--------------------------------|---------------|
| cg19135245 | 0.272388772 | 0.999997557                    | 0.004258499   |
| cg07733851 | 0.279542254 | 0.999997557                    | 0.02357243    |
| cg15910486 | 0.292918216 | 0.999997557                    | 0.004537478   |
| cg01967637 | 0.338536262 | 0.999997557                    | 0.003919932   |
| cg17860381 | 0.357836419 | 0.999997557                    | 0.000876506   |
| cg18849621 | 0.379245855 | 0.999997557                    | 0.002552033   |
| cg21702128 | 0.406504887 | 0.999997557                    | − 0.001070247 |
| cg13764763 | 0.454791344 | 0.999997557                    | 0.015622476   |
| cg00629244 | 0.503885658 | 0.999997557                    | − 0.00246556  |
| cg14939152 | 0.504120134 | 0.999997557                    | 0.000577132   |
| cg27122725 | 0.529860939 | 0.999997557                    | 0.006029979   |
| cg14558428 | 0.531421634 | 0.999997557                    | 0.001417758   |
| cg08818984 | 0.551707805 | 0.999997557                    | − 0.030134797 |
| cg24026230 | 0.564518425 | 0.999997557                    | 0.002507375   |
| cg03906910 | 0.630630252 | 0.999997557                    | − 0.02119966  |
| cg13648501 | 0.652981749 | 0.999997557                    | 0.001717513   |
| cg16335926 | 0.740313284 | 0.999997557                    | − 0.001532178 |
| cg26720913 | 0.743323678 | 0.999997557                    | − 0.017368038 |
| cg17342132 | 0.818325933 | 0.999997557                    | 0.011875955   |
| cg18718518 | 0.88056981  | 0.999997557                    | 0.004555236   |
| cg22402730 | 0.908119964 | 0.999997557                    | − 0.000126521 |
| cg15645634 | 0.908177372 | 0.999997557                    | − 0.001196809 |
| cg23776787 | 0.933952752 | 0.999997557                    | − 0.00580295  |
| cg11152298 | 0.951420262 | 0.999997557                    | 0.000520728   |
| cg18998365 | 0.961116448 | 0.999997557                    | 0.001743816   |

Δβ = mean β (treatment as usual) – mean β (cognitive behavioral therapy)

TSS transcription start site, UTR untranslated region

<sup>a</sup>Adjusted for multiple testing [45]**Table 8** Top 100 differentially methylated probes according to baseline depression (BDI-II)

| CpG        | <i>p</i>    | Adjusted <i>p</i> <sup>a</sup> | Gene          | Gene region | Δβ            |
|------------|-------------|--------------------------------|---------------|-------------|---------------|
| cg01656717 | 5.43E-05    | 0.985858571                    | <i>WWP2</i>   | Body        | 0.020713379   |
| cg06022376 | 5.62E-05    | 0.985858571                    | <i>CACTIN</i> | Body        | 0.031934062   |
| cg01120173 | 5.91E-05    | 0.985858571                    | <i>ZNF232</i> | 5'UTR       | − 0.032894902 |
| cg24732447 | 8.42E-05    | 0.985858571                    | <i>OSTM1</i>  | TSS1500     | − 0.040891939 |
| cg17402103 | 9.76E-05    | 0.985858571                    |               |             | 0.044389084   |
| cg10276665 | 0.000102293 | 0.985858571                    | <i>PHF20</i>  | 5'UTR       | − 0.053135525 |
| cg23119960 | 0.000108933 | 0.985858571                    | <i>TCF12</i>  | TSS1500     | 0.019411015   |

**Table 8** Top 100 differentially methylated probes according to baseline depression (BDI-II) (Continued)

| CpG        | <i>p</i>    | Adjusted <i>p</i> <sup>a</sup> | Gene                | Gene region | $\Delta\beta$ |
|------------|-------------|--------------------------------|---------------------|-------------|---------------|
| cg07639472 | 0.000110211 | 0.985858571                    | <i>GABARAP</i>      | TSS200      | 0.009595275   |
| cg14522236 | 0.000112046 | 0.985858571                    |                     |             | − 0.049713856 |
| cg16561657 | 0.000150143 | 0.985858571                    |                     |             | − 0.055819652 |
| cg21014120 | 0.00015174  | 0.985858571                    | <i>ICA1L</i>        | TSS200      | − 0.006901363 |
| cg02965870 | 0.000158156 | 0.985858571                    | <i>NEDD4</i>        | 1stExon     | − 0.005222348 |
| cg19817882 | 0.000171789 | 0.985858571                    | <i>LEFTY1</i>       | Body        | 0.034762224   |
| cg02644616 | 0.000173319 | 0.985858571                    |                     |             | − 0.00792669  |
| cg00369151 | 0.000179443 | 0.985858571                    | <i>PIP4K2A</i>      | Body        | − 0.036713932 |
| cg24954665 | 0.000194603 | 0.985858571                    |                     |             | − 0.018476691 |
| cg08217452 | 0.000200085 | 0.985858571                    |                     |             | 0.062010845   |
| cg22796353 | 0.000209579 | 0.985858571                    |                     |             | − 0.06798597  |
| cg05636467 | 0.000213261 | 0.985858571                    | <i>EBF3</i>         | Body        | 0.058972943   |
| cg01870580 | 0.000213772 | 0.985858571                    | <i>SGCD</i>         | Body        | − 0.033578993 |
| cg04167481 | 0.000227738 | 0.985858571                    | <i>LRRC6</i>        | Body        | − 0.02281918  |
| cg07010552 | 0.000233    | 0.985858571                    | <i>CHRNA1</i>       | Body        | 0.03251269    |
| cg09877950 | 0.000238965 | 0.985858571                    | <i>SLC4A10</i>      | Body        | − 0.050949676 |
| cg08548444 | 0.000241925 | 0.985858571                    |                     |             | 0.058594659   |
| cg22870344 | 0.000242958 | 0.985858571                    | <i>ATP5B</i>        | TSS200      | 0.040824173   |
| cg16692066 | 0.000251174 | 0.985858571                    | <i>FNDC7</i>        | Body        | − 0.027886693 |
| cg03781315 | 0.00025551  | 0.985858571                    | <i>AHCY</i>         | Body        | − 0.020798236 |
| cg18303019 | 0.000261834 | 0.985858571                    | <i>TXNRD1</i>       | TSS1500     | − 0.03100706  |
| cg07381391 | 0.000267381 | 0.985858571                    |                     |             | 0.203102371   |
| cg17115402 | 0.000269335 | 0.985858571                    | <i>CDR2L</i>        | Body        | − 0.020519014 |
| cg23788051 | 0.000272662 | 0.985858571                    |                     |             | 0.034154924   |
| cg15234197 | 0.000277725 | 0.985858571                    |                     |             | 0.09308691    |
| cg22521539 | 0.000282937 | 0.985858571                    |                     |             | 0.049648454   |
| cg25157095 | 0.000284638 | 0.985858571                    | <i>RIPK4</i>        | Body        | 0.029861946   |
| cg25464078 | 0.000290016 | 0.985858571                    | <i>PPTC7</i>        | Body        | 0.041450584   |
| cg24667213 | 0.000295285 | 0.985858571                    |                     |             | 0.021353983   |
| cg03716908 | 0.00029717  | 0.985858571                    |                     |             | 0.036164552   |
| cg11747082 | 0.000319919 | 0.985858571                    | <i>GPR33</i>        | TSS1500     | − 0.043309753 |
| cg08446512 | 0.000321548 | 0.985858571                    | <i>MIR548Q</i>      | Body        | − 0.057652312 |
| cg10239816 | 0.000321981 | 0.985858571                    | <i>GOT1</i>         | TSS200      | 0.010999285   |
| cg24632014 | 0.000329696 | 0.985858571                    | <i>LOC100189589</i> | Body        | 0.033737209   |
| cg14255237 | 0.000331265 | 0.985858571                    | <i>SARDH</i>        | Body        | 0.068885565   |
| cg01874640 | 0.000341932 | 0.985858571                    | <i>HGD</i>          | ExonBnd     | − 0.027385445 |
| cg12308055 | 0.000342843 | 0.985858571                    | <i>VAC14</i>        | Body        | 0.025670901   |
| cg13747435 | 0.000353254 | 0.985858571                    | <i>AK1</i>          | Body        | 0.02153699    |
| cg26287679 | 0.000353404 | 0.985858571                    | <i>MYBL1</i>        | Body        | − 0.036514738 |
| cg27305222 | 0.000359452 | 0.985858571                    |                     |             | − 0.040634637 |
| cg09694986 | 0.000364186 | 0.985858571                    | <i>SNTB1</i>        | Body        | − 0.041411009 |
| cg04928577 | 0.000370129 | 0.985858571                    |                     |             | − 0.069549039 |
| cg02059927 | 0.000376777 | 0.985858571                    |                     |             | 0.045567447   |
| cg19553615 | 0.000379462 | 0.985858571                    | <i>CRTC3</i>        | Body        | 0.021785594   |

**Table 8** Top 100 differentially methylated probes according to baseline depression (BDI-II) (Continued)

| CpG        | <i>p</i>    | Adjusted <i>p</i> <sup>a</sup> | Gene            | Gene region | $\Delta\beta$ |
|------------|-------------|--------------------------------|-----------------|-------------|---------------|
| cg06214427 | 0.000382521 | 0.985858571                    | <i>MYO1A</i>    | Body        | − 0.027829513 |
| cg14609960 | 0.000388595 | 0.985858571                    | <i>PITRM1</i>   | Body        | − 0.030711115 |
| cg07814876 | 0.000392304 | 0.985858571                    | <i>GGPS1</i>    | 5'UTR       | 0.02176791    |
| cg03656020 | 0.000394532 | 0.985858571                    | <i>VGF</i>      | 3'UTR       | 0.02323939    |
| cg16977720 | 0.000414373 | 0.985858571                    | <i>TRABD2A</i>  | Body        | − 0.015144172 |
| cg11173076 | 0.00041489  | 0.985858571                    | <i>ART1</i>     | TSS200      | 0.051579054   |
| cg11407226 | 0.000427414 | 0.985858571                    |                 |             | 0.052957159   |
| cg24676514 | 0.000428063 | 0.985858571                    |                 |             | 0.007114356   |
| cg24353217 | 0.000430568 | 0.985858571                    | <i>MYL2</i>     | Body        | 0.048161701   |
| cg13022689 | 0.000438434 | 0.985858571                    |                 |             | − 0.014053374 |
| cg08013270 | 0.000452709 | 0.985858571                    | <i>EMX1</i>     | Body        | 0.009802214   |
| cg10486455 | 0.000457554 | 0.985858571                    | <i>WDR46</i>    | Body        | − 0.071572335 |
| cg08824610 | 0.000457605 | 0.985858571                    | <i>SCN3B</i>    | Body        | 0.032374425   |
| cg23934072 | 0.00046317  | 0.985858571                    | <i>KIF21B</i>   | 3'UTR       | 0.072005944   |
| cg08882432 | 0.000492053 | 0.985858571                    | <i>CCDC171</i>  | Body        | − 0.06825542  |
| cg19075081 | 0.000509177 | 0.985858571                    | <i>MTSS1L</i>   | Body        | 0.034977378   |
| cg14940449 | 0.000513204 | 0.985858571                    | <i>HGS</i>      | TSS200      | − 0.004493762 |
| cg27644292 | 0.000535008 | 0.985858571                    | <i>SNRPN</i>    | 5'UTR       | − 0.043063624 |
| cg13277044 | 0.000537047 | 0.985858571                    |                 |             | − 0.028669058 |
| cg10313065 | 0.000547596 | 0.985858571                    |                 |             | 0.027390264   |
| cg27483342 | 0.000549745 | 0.985858571                    |                 |             | − 0.035483152 |
| cg00167525 | 0.00054993  | 0.985858571                    |                 |             | − 0.044404069 |
| cg02624701 | 0.000556261 | 0.985858571                    | <i>SLC17A7</i>  | Body        | − 0.023735747 |
| cg24488506 | 0.000559886 | 0.985858571                    | <i>FOSL1</i>    | 1stExon     | − 0.005249818 |
| cg10894284 | 0.000567688 | 0.985858571                    | <i>SPATS2</i>   | Body        | − 0.05283773  |
| cg00045787 | 0.0005679   | 0.985858571                    | <i>SNTB2</i>    | Body        | 0.021702408   |
| cg22379574 | 0.000572536 | 0.985858571                    | <i>TPT1</i>     | TSS200      | 0.002542434   |
| cg09381162 | 0.000579437 | 0.985858571                    | <i>ANXA13</i>   | Body        | − 0.038107869 |
| cg10562399 | 0.000581216 | 0.985858571                    | <i>SNRPG</i>    | Body        | 0.049683592   |
| cg17422878 | 0.000584164 | 0.985858571                    |                 |             | − 0.01955572  |
| cg16460816 | 0.000592284 | 0.985858571                    | <i>IFT140</i>   | Body        | 0.016906513   |
| cg22647874 | 0.000594316 | 0.985858571                    | <i>FAM192A</i>  | 5'UTR       | − 0.01781755  |
| cg04157647 | 0.000594803 | 0.985858571                    | <i>CD27-AS1</i> | Body        | − 0.068075731 |
| cg14436051 | 0.000595366 | 0.985858571                    | <i>PRR26</i>    | Body        | − 0.018081196 |
| cg11629443 | 0.000598589 | 0.985858571                    | <i>TRIM27</i>   | 1stExon     | 0.005616034   |
| cg03163982 | 0.00059979  | 0.985858571                    |                 |             | − 0.008328044 |
| cg11475558 | 0.000600783 | 0.985858571                    | <i>TNS1</i>     | Body        | 0.028042851   |
| cg18014277 | 0.000608293 | 0.985858571                    | <i>APBB1IP</i>  | 3'UTR       | − 0.016579214 |
| cg02597373 | 0.000619621 | 0.985858571                    | <i>UNC13D</i>   | Body        | 0.05993223    |
| cg23123838 | 0.000622213 | 0.985858571                    | <i>MTA1</i>     | TSS200      | 0.023497892   |
| cg03278573 | 0.000627109 | 0.985858571                    | <i>DAP</i>      | Body        | − 0.064990789 |
| cg15674937 | 0.000643134 | 0.985858571                    |                 |             | 0.073468304   |
| cg01126532 | 0.000643521 | 0.985858571                    |                 |             | − 0.081499283 |
| cg04736676 | 0.000662804 | 0.985858571                    | <i>MCM3AP</i>   | TSS1500     | 0.012334518   |

**Table 8** Top 100 differentially methylated probes according to baseline depression (BDI-II) (Continued)

| CpG        | <i>p</i>    | Adjusted <i>p</i> <sup>a</sup> | Gene           | Gene region | $\Delta\beta$ |
|------------|-------------|--------------------------------|----------------|-------------|---------------|
| cg11240430 | 0.000664374 | 0.985858571                    | <i>ANKRD16</i> | 5'UTR       | 0.017463959   |
| cg00154646 | 0.000664385 | 0.985858571                    |                |             | − 0.022644413 |
| cg06434997 | 0.000669569 | 0.985858571                    | <i>FBXO5</i>   | 5'UTR       | 0.023162548   |
| cg02030350 | 0.00067758  | 0.985858571                    |                | Body        | − 0.030538262 |
| cg13357903 | 0.000693814 | 0.985858571                    | <i>MIA3</i>    | TSS1500     | 0.012988536   |

 $\Delta\beta$  = mean  $\beta$  (severely depressed) – mean  $\beta$  (mildly depressed)

TSS transcription start site, UTR untranslated region

<sup>a</sup>Adjusted for multiple testing [46]**Table 9** Top 100 differentially methylated probes according to baseline anxiety (BAI)

| CpG        | <i>p</i>    | Adjusted <i>p</i> <sup>a</sup> | Gene             | Gene region | $\Delta\beta$ |
|------------|-------------|--------------------------------|------------------|-------------|---------------|
| cg06513375 | 1.01E-06    | 0.77590274                     | <i>ZNF251</i>    | Body        | − 0.106421132 |
| cg19573881 | 5.11E-06    | 0.998778059                    |                  |             | 0.083994741   |
| cg00117018 | 1.40E-05    | 0.998778059                    | <i>ZNF251</i>    | Body        | − 0.13038673  |
| cg11602361 | 3.18E-05    | 0.998778059                    | <i>FYN</i>       | 5'UTR       | − 0.045924181 |
| cg21641920 | 3.80E-05    | 0.998778059                    | <i>RBM33</i>     | Body        | − 0.056092107 |
| cg13511253 | 4.12E-05    | 0.998778059                    | <i>MAPK4</i>     | 5'UTR       | − 0.06921116  |
| cg11674381 | 4.68E-05    | 0.998778059                    |                  |             | − 0.030888002 |
| cg00115113 | 5.01E-05    | 0.998778059                    | <i>LINC00483</i> | Body        | 0.027732238   |
| cg21918548 | 5.84E-05    | 0.998778059                    | <i>ZNF251</i>    | Body        | − 0.100935223 |
| cg01519784 | 5.87E-05    | 0.998778059                    |                  |             | − 0.025857817 |
| cg07081372 | 6.58E-05    | 0.998778059                    | <i>TMX1</i>      | Body        | 0.020743268   |
| cg26293081 | 7.19E-05    | 0.998778059                    | <i>TNS3</i>      | Body        | 0.039738087   |
| cg06626791 | 7.25E-05    | 0.998778059                    | <i>CCNE2</i>     | 5'UTR       | 0.012276869   |
| cg04788249 | 7.26E-05    | 0.998778059                    | <i>ATG7</i>      | 5'UTR       | 0.003609404   |
| cg08049441 | 7.76E-05    | 0.998778059                    | <i>RPL32P3</i>   | Body        | − 0.024015531 |
| cg10731606 | 8.45E-05    | 0.998778059                    | <i>AGBL3</i>     | TSS200      | 0.031982023   |
| cg02335517 | 0.000117192 | 0.998778059                    | <i>IL6</i>       | Body        | − 0.013920705 |
| cg12379948 | 0.00011944  | 0.998778059                    | <i>WNT3</i>      | TSS1500     | 0.007283815   |
| cg13242754 | 0.000127218 | 0.998778059                    | <i>C14orf101</i> | Body        | − 0.015166989 |
| cg06245967 | 0.000130491 | 0.998778059                    | <i>BANP</i>      | 5'UTR       | − 0.029761211 |
| cg21643916 | 0.000138817 | 0.998778059                    | <i>PRKAR1B</i>   | Body        | − 0.013338272 |
| cg22500132 | 0.000147833 | 0.998778059                    | <i>MUC1</i>      | TSS200      | 0.00823408    |
| cg24555816 | 0.000150316 | 0.998778059                    |                  |             | 0.058918432   |
| cg02893361 | 0.000160529 | 0.998778059                    | <i>PIAS1</i>     | Body        | − 0.030954487 |
| cg12906188 | 0.000164316 | 0.998778059                    | <i>RGS4</i>      | Body        | 0.008487123   |
| cg05524951 | 0.000170319 | 0.998778059                    |                  |             | − 0.012679223 |
| cg14122980 | 0.000170584 | 0.998778059                    | <i>PTPRD</i>     | 5'UTR       | − 0.023320139 |
| cg13449967 | 0.000178787 | 0.998778059                    | <i>ATG2A</i>     | Body        | 0.029533776   |
| cg17231980 | 0.000185655 | 0.998778059                    |                  |             | − 0.013659095 |
| cg04657000 | 0.000189668 | 0.998778059                    | <i>FYN</i>       | 5'UTR       | − 0.012205559 |
| cg18612255 | 0.000205249 | 0.998778059                    |                  |             | 0.012801625   |
| cg22063222 | 0.000229138 | 0.998778059                    |                  |             | − 0.010538791 |
| cg23760165 | 0.000231842 | 0.998778059                    | <i>FADS2</i>     | TSS1500     | 0.00669263    |
| cg24531534 | 0.000237063 | 0.998778059                    | <i>LOXL2</i>     | Body        | 0.102423109   |
| cg15745507 | 0.000240352 | 0.998778059                    |                  |             | 0.039058624   |
| cg05731717 | 0.000243608 | 0.998778059                    |                  |             | − 0.038853941 |
| cg16888838 | 0.000245704 | 0.998778059                    | <i>KIAA1549</i>  | 3'UTR       | − 0.021553937 |
| cg17190403 | 0.000249731 | 0.998778059                    | <i>C6orf211</i>  | Body        | 0.029783554   |
| cg18298090 | 0.000274478 | 0.998778059                    | <i>ETV2</i>      | TSS1500     | − 0.035320986 |

**Table 9** Top 100 differentially methylated probes according to baseline anxiety (BAI) (Continued)

| CpG        | <i>p</i>    | Adjusted <i>p</i> <sup>a</sup> | Gene                | Gene region | $\Delta\beta$ |
|------------|-------------|--------------------------------|---------------------|-------------|---------------|
| cg11341317 | 0.000285303 | 0.998778059                    |                     |             | − 0.032941327 |
| cg15264808 | 0.000285946 | 0.998778059                    | <i>CENPN</i>        | 5'UTR       | 0.012161477   |
| cg21025551 | 0.000290064 | 0.998778059                    | <i>ADRBK2</i>       | TSS200      | 0.008238703   |
| cg15872329 | 0.000304128 | 0.998778059                    | <i>BLOC1S2</i>      | Body        | 0.010747948   |
| cg26594377 | 0.000311806 | 0.998778059                    | <i>EFCAB11</i>      | 5'UTR       | 0.007818559   |
| cg27191554 | 0.000311819 | 0.998778059                    | <i>NOTCH3</i>       | Body        | 0.016376385   |
| cg25899954 | 0.000314492 | 0.998778059                    |                     |             | − 0.015053877 |
| cg09602803 | 0.000326585 | 0.998778059                    |                     |             | 0.052616473   |
| cg23462514 | 0.000333695 | 0.998778059                    | <i>RNF212</i>       | TSS200      | − 0.085842379 |
| cg18193440 | 0.000336288 | 0.998778059                    | <i>TAF1L</i>        | 1stExon     | 0.071586528   |
| cg09398891 | 0.000343573 | 0.998778059                    |                     |             | − 0.017821675 |
| cg08949296 | 0.000351898 | 0.998778059                    | <i>JPH1</i>         | 1stExon     | 0.008914397   |
| cg21943599 | 0.000355323 | 0.998778059                    | <i>C1orf125</i>     | TSS1500     | − 0.012630326 |
| cg04322378 | 0.000356074 | 0.998778059                    | <i>LINC01258</i>    | TSS200      | 0.036276539   |
| cg13921204 | 0.000358982 | 0.998778059                    | <i>SEC61A2</i>      | TSS200      | 0.004130793   |
| cg07346187 | 0.000360053 | 0.998778059                    | <i>ZC3H12D</i>      | Body        | 0.008900888   |
| cg11832804 | 0.000361177 | 0.998778059                    | <i>TERT</i>         | Body        | − 0.006660909 |
| cg04899629 | 0.00036328  | 0.998778059                    | <i>LOR2C3</i>       | TSS1500     | − 0.067707778 |
| cg01985858 | 0.000364399 | 0.998778059                    | <i>OBFC2B</i>       | TSS1500     | 0.012234912   |
| cg03851648 | 0.000366413 | 0.998778059                    | <i>PHC2</i>         | Body        | − 0.103038845 |
| cg11102724 | 0.000382353 | 0.998778059                    |                     |             | 0.200746665   |
| cg18570658 | 0.000387535 | 0.998778059                    | <i>COL4A2</i>       | Body        | − 0.06295432  |
| cg24942330 | 0.000389195 | 0.998778059                    | <i>ASAHI</i>        | TSS1500     | 0.005198328   |
| cg07571142 | 0.00039639  | 0.998778059                    | <i>C10orf99</i>     | 3'UTR       | − 0.022383608 |
| cg14405643 | 0.000402335 | 0.998778059                    | <i>IER5L</i>        | 3'UTR       | 0.026828829   |
| cg13147522 | 0.000402551 | 0.998778059                    | <i>SAP53</i>        | TSS200      | 0.011988911   |
| cg15417944 | 0.000405638 | 0.998778059                    | <i>RBM44</i>        | 5'UTR       | − 0.03261725  |
| cg00616952 | 0.000409576 | 0.998778059                    | <i>SIPA1L3</i>      | Body        | − 0.019003771 |
| cg23166923 | 0.000410512 | 0.998778059                    | <i>PMPCA</i>        | 1stExon     | 0.008004775   |
| cg13297582 | 0.000411378 | 0.998778059                    | <i>LDLRAD4</i>      | 5'UTR       | − 0.092481987 |
| cg00962271 | 0.000413861 | 0.998778059                    |                     |             | − 0.042309368 |
| cg11640106 | 0.000416865 | 0.998778059                    | <i>LOC101929194</i> | Body        | − 0.016168103 |
| cg06981781 | 0.000418137 | 0.998778059                    | <i>EGF</i>          | Body        | − 0.011108508 |
| cg24146773 | 0.000418853 | 0.998778059                    | <i>SH3BGR</i>       | 1stExon     | − 0.083744695 |
| cg23579746 | 0.000438092 | 0.998778059                    | <i>FCRLB</i>        | TSS1500     | − 0.026251069 |
| cg09819772 | 0.000438692 | 0.998778059                    |                     |             | − 0.019080858 |
| cg06630983 | 0.000440009 | 0.998778059                    | <i>PPM1F</i>        | Body        | − 0.013620731 |
| cg09207053 | 0.000444686 | 0.998778059                    | <i>PCDHGA11</i>     | TSS200      | 0.021510517   |
| cg11833983 | 0.000447858 | 0.998778059                    | <i>KANSL2</i>       | Body        | − 0.020712784 |
| cg05675803 | 0.000455891 | 0.998778059                    | <i>C6orf52</i>      | Body        | 0.006716337   |
| cg03265692 | 0.000455941 | 0.998778059                    | <i>ATAD1</i>        | TSS1500     | 0.010548663   |
| cg11463903 | 0.000458655 | 0.998778059                    | <i>ING5</i>         | TSS1500     | 0.015381351   |
| cg03211481 | 0.00046527  | 0.998778059                    | <i>DNAJC1</i>       | Body        | − 0.022278955 |
| cg17714799 | 0.000472182 | 0.998778059                    | <i>CASP6</i>        | TSS1500     | 0.018907097   |
| cg20034712 | 0.000482406 | 0.998778059                    | <i>ZNF836</i>       | TSS1500     | − 0.060359087 |
| cg11554391 | 0.000485943 | 0.998778059                    | <i>AHRR</i>         | Body        | 0.014764295   |
| cg06166863 | 0.000490293 | 0.998778059                    | <i>PNN</i>          | TSS200      | 0.007284371   |
| cg26321013 | 0.000491445 | 0.998778059                    | <i>WIPF2</i>        | 1stExon     | 0.018566869   |
| cg16261619 | 0.000495054 | 0.998778059                    | <i>ZBPB</i>         | TSS200      | − 0.049720147 |



**Table 9** Top 100 differentially methylated probes according to baseline anxiety (BAI) (Continued)

| CpG        | <i>p</i>    | Adjusted <i>p</i> <sup>a</sup> | Gene             | Gene region | $\Delta\beta$ |
|------------|-------------|--------------------------------|------------------|-------------|---------------|
| cg06871884 | 0.000495095 | 0.998778059                    | <i>LINC00963</i> | Body        | 0.008107368   |
| cg16333300 | 0.000496236 | 0.998778059                    | <i>TECTA</i>     | Body        | − 0.023279226 |
| cg21848211 | 0.000497682 | 0.998778059                    |                  |             | − 0.019228316 |
| cg16287252 | 0.00050262  | 0.998778059                    | <i>GLT1D1</i>    | Body        | − 0.059937553 |
| cg15568778 | 0.000504593 | 0.998778059                    |                  |             | − 0.009418856 |
| cg15247039 | 0.000514355 | 0.998778059                    |                  |             | − 0.026521804 |
| cg04800443 | 0.000518233 | 0.998778059                    |                  |             | 0.034208928   |
| cg12937337 | 0.000519654 | 0.998778059                    | <i>PTEN</i>      | 5'UTR       | − 0.020668502 |
| cg05308125 | 0.000534128 | 0.998778059                    |                  |             | − 0.017025401 |
| cg13267264 | 0.000538758 | 0.998778059                    | <i>PRDM14</i>    | TSS200      | 0.023761421   |
| cg06610641 | 0.000538794 | 0.998778059                    | <i>ZNF527</i>    | TSS1500     | 0.019166156   |
| cg16642284 | 0.00053992  | 0.998778059                    | <i>FOXI2</i>     | TSS200      | 0.019871733   |

 $\Delta\beta$  = mean  $\beta$  (severely anxious) – mean  $\beta$  (mildly anxious)

TSS transcription start site, UTR untranslated region

<sup>a</sup>Adjusted for multiple testing [46]**Table 10** Differential methylation of candidate genes according to baseline depression (BDI-II)

| Gene                  | $\Delta\beta$ | 95% CI             | <i>p</i> |
|-----------------------|---------------|--------------------|----------|
| <i>NR3C1</i>          | 0.002         | − 0.006 to 0.011   | 0.647    |
| <i>NR3C1</i> Promoter | 0.006         | − 0.005 to 0.020   | 0.2093   |
| <i>SLC6A4</i>         | 0.004         | − 0.022 to 0.028   | 0.647    |
| <i>OXTR</i>           | 0.003         | − 0.009 to 0.010   | 0.5123   |
| <i>NR3C2</i>          | − 0.002       | − 0.012 to 0.007   | 0.647    |
| <i>MEST</i>           | 0.009         | − 0.002 to 0.018   | 0.1264   |
| <i>MEG3</i>           | 0.007         | − 0.008 to 0.0120  | 0.2921   |
| <i>IFG2</i>           | − 0.006       | − 0.035 to 0.018   | 0.6005   |
| <i>HSD11B1</i>        | − 0.002       | − 0.022 to 0.017   | 0.7938   |
| <i>HSD11B2</i>        | 0.004         | − 0.005 to 0.009   | 0.2093   |
| <i>H19</i>            | 0.016         | − 0.011 to 0.039   | 0.2624   |
| <i>CRHR1</i>          | 0.008         | − 0.016 to 0.0221  | 0.3575   |
| <i>CRHR2</i>          | 0.005         | − 0.014 to 0.0246  | 0.647    |
| <i>CRHRBP</i>         | 0.011         | − 0.0262 to 0.0372 | 0.5556   |
| <i>CRH</i>            | − 0.006       | − 0.017 to 0.009   | 0.3237   |
| <i>BDNF</i>           | 0.003         | − 0.001 to 0.010   | 0.1641   |
| <i>FKBP5</i>          | 0.003         | − 0.023 to 0.031   | 0.7414   |

 $\Delta\beta$  = mean  $\beta$  (severely depressed) – mean  $\beta$  (mildly depressed)**Table 11** Probes in candidate gene analysis showing differential methylation according to baseline depression (BDI-II) at uncorrected *p* < 0.01

| CpG        | <i>p</i>    | Adjusted <i>p</i> <sup>a</sup> | Gene         | Gene region | $\Delta\beta$ |
|------------|-------------|--------------------------------|--------------|-------------|---------------|
| cg17578833 | 0.002812934 | 0.985858571                    | <i>CRH</i>   | TSS1500     | − 0.055448723 |
| cg04137760 | 0.00427371  | 0.985858571                    | <i>FKBP5</i> | 5'UTR       | − 0.028541521 |
| cg08077673 | 0.007257559 | 0.985858571                    | <i>MEST</i>  | 5'UTR       | 0.008859633   |
| cg07583420 | 0.00759847  | 0.985858571                    | <i>IGF2</i>  | Body        | 0.00580552    |
| cg13167664 | 0.009158888 | 0.985858571                    | <i>IGF2</i>  | Body        | 0.003859675   |

 $\Delta\beta$  = mean  $\beta$  (severely depressed) – mean  $\beta$  (mildly depressed)

TSS transcription start site, UTR untranslated region

<sup>a</sup>Adjusted for multiple testing [45]

**Table 12** Differential methylation of candidate genes according to baseline anxiety (BAI)

| Gene                  | $\Delta\beta$ | 95% CI            | <i>p</i> |
|-----------------------|---------------|-------------------|----------|
| <i>NR3C1</i>          | − 0.006       | − 0.013 to 0.004  | 0.2382   |
| <i>NR3C1</i> Promoter | 0.008         | − 0.001 to 0.019  | 0.0817   |
| <i>SLC6A4</i>         | 0.005         | − 0.022 to 0.028  | 0.5699   |
| <i>OXTR</i>           | − 0.004       | − 0.015 to 0.005  | 0.3301   |
| <i>NR3C2</i>          | 0.004         | − 0.007 to 0.011  | 0.4411   |
| <i>MEST</i>           | 0.013         | 0.001 to 0.023    | 0.01965  |
| <i>MEG3</i>           | 0.012         | − 0.0005 to 0.025 | 0.06983  |
| <i>IFG2</i>           | − 0.004       | − 0.028 to 0.026  | 0.7135   |
| <i>HSD11B1</i>        | 0.009         | − 0.008 to 0.027  | 0.2667   |
| <i>HSD11B2</i>        | 0.005         | − 0.003 to 0.012  | 0.11     |
| <i>H19</i>            | 0.014         | − 0.009 to 0.039  | 0.2382   |
| <i>CRHR1</i>          | 0.003         | − 0.015 to 0.021  | 0.6163   |
| <i>CRHR2</i>          | 0.001         | − 0.018 to 0.024  | 0.9734   |
| <i>CRHRBP</i>         | 0.016         | − 0.021 to 0.045  | 0.4411   |
| <i>CRH</i>            | − 0.007       | − 0.019 to 0.006  | 0.402    |
| <i>BDNF</i>           | 0.007         | − 0.0007 to 0.011 | 0.0817   |
| <i>FKBP5</i>          | 0.005         | − 0.003 to 0.014  | 0.145    |

 $\Delta\beta$  = mean  $\beta$  (severely anxious) – mean  $\beta$  (mildly anxious)**Table 13** Differential methylation according to baseline anxiety (BAI) (promoter-associated *NR3C1* probes)

| CpG        | <i>p</i>    | Adjusted <i>p</i> <sup>a</sup> | Gene region | $\Delta\beta$ |
|------------|-------------|--------------------------------|-------------|---------------|
| cg07515400 | 0.019543236 | 0.998778059                    | TSS1500     | 0.008757408   |
| cg22402730 | 0.034941814 | 0.998778059                    | TSS1500     | 0.007846464   |
| cg18068240 | 0.074595651 | 0.998778059                    | 5'UTR       | 0.003513867   |
| cg00629244 | 0.075603387 | 0.998778059                    | TSS200      | 0.005153991   |
| cg21209684 | 0.095644074 | 0.998778059                    | 5'UTR       | 0.005819462   |
| cg17860381 | 0.172486506 | 0.998778059                    | 5'UTR       | − 0.007857672 |
| cg18849621 | 0.181055644 | 0.998778059                    | TSS1500     | 0.007722928   |
| cg26720913 | 0.22868997  | 0.998778059                    | 1stExon     | 0.071542284   |
| cg16335926 | 0.238092713 | 0.998778059                    | TSS1500     | 0.002021547   |
| cg24026230 | 0.24577813  | 0.998778059                    | 5'UTR       | 0.005938353   |
| cg18019515 | 0.245786808 | 0.998778059                    | TSS200      | 0.001549976   |
| cg23776787 | 0.295148027 | 0.998778059                    | 1stExon     | 0.055157644   |
| cg11152298 | 0.296050346 | 0.998778059                    | TSS200      | 0.003074345   |
| cg17342132 | 0.318902496 | 0.998778059                    | Body        | − 0.021285299 |
| cg27122725 | 0.347102162 | 0.998778059                    | 5'UTR       | 0.035263092   |
| cg10847032 | 0.355174482 | 0.998778059                    | TSS1500     | − 0.000186142 |
| cg21702128 | 0.358888726 | 0.998778059                    | TSS1500     | 0.003153478   |
| cg26464411 | 0.373131972 | 0.998778059                    | TSS1500     | 0.008722328   |
| cg18998365 | 0.436272234 | 0.998778059                    | 5'UTR       | 0.005641782   |
| cg06968181 | 0.486353571 | 0.998778059                    | TSS1500     | 0.004702526   |
| cg03906910 | 0.524841281 | 0.998778059                    | 1stExon     | 0.051231481   |
| cg14939152 | 0.572528468 | 0.998778059                    | 5'UTR       | − 0.003292981 |
| cg04111177 | 0.59537643  | 0.998778059                    | 5'UTR       | 0.002463121   |

**Table 13** Differential methylation according to baseline anxiety (BAI) (promoter-associated *NR3C1* probes) (Continued)

| CpG        | <i>p</i>    | Adjusted <i>p</i> <sup>a</sup> | Gene region | Δβ           |
|------------|-------------|--------------------------------|-------------|--------------|
| cg06952416 | 0.665139695 | 0.998778059                    | 5'UTR       | 0.036482196  |
| cg08818984 | 0.673915225 | 0.998778059                    | 1stExon     | 0.043838345  |
| cg13648501 | 0.723256995 | 0.998778059                    | 5'UTR       | 0.008987005  |
| cg19135245 | 0.779311238 | 0.998778059                    | TSS1500     | 0.001611098  |
| cg07733851 | 0.816930585 | 0.998778059                    | 5'UTR       | 0.029161123  |
| cg15645634 | 0.864608392 | 0.998778059                    | 5'UTR       | −0.002973062 |
| cg01967637 | 0.911624523 | 0.999388148                    | 5'UTR       | −0.004461401 |
| cg14558428 | 0.913633924 | 0.999388148                    | 5'UTR       | 0.000461939  |
| cg18718518 | 0.937744615 | 0.999400864                    | TSS1500     | 0.023943315  |
| cg13764763 | 0.939306554 | 0.999410992                    | TSS1500     | 0.012472219  |
| cg15910486 | 0.975291537 | 0.999632341                    | 5'UTR       | −0.003768688 |

Δβ = mean β (severely anxious) - mean β (mildly anxious)

TSS transcription start site, UTR untranslated region

<sup>a</sup>Adjusted for multiple testing [45]

**Table 14** Probes in candidate gene analysis showing differential methylation according to baseline anxiety (BAI) at uncorrected  $p < 0.01$ 

| CpG        | <i>p</i>    | Adjusted <i>p</i> <sup>a</sup> | Gene           | Gene region | Δβ           |
|------------|-------------|--------------------------------|----------------|-------------|--------------|
| cg26880525 | 0.00670039  | 0.998778059                    | <i>HSD11B1</i> | 5'UTR       | −0.07833178  |
| cg07704699 | 0.007191379 | 0.998778059                    | <i>BDNF</i>    | Body        | 0.026796044  |
| cg13670288 | 0.007464434 | 0.998778059                    | <i>IGF2</i>    | Body        | −0.003490477 |
| cg23273257 | 0.009092701 | 0.998778059                    | <i>NR3C1</i>   | 3'UTR       | −0.014724328 |

Δβ = mean β (severely anxious) - mean β (mildly anxious)

TSS transcription start site, UTR untranslated region

<sup>a</sup>Adjusted for multiple testing [45]

**Table 15** Differential methylation according to baseline depression (BDI-II) (promoter-associated *NR3C1* probes)

| CpG        | <i>p</i>    | Adjusted <i>p</i> <sup>a</sup> | Gene region | Δβ           |
|------------|-------------|--------------------------------|-------------|--------------|
| cg22402730 | 0.09232524  | 0.985858571                    | TSS1500     | 0.007914958  |
| cg07515400 | 0.14983598  | 0.985858571                    | TSS1500     | 0.00381786   |
| cg18849621 | 0.155838866 | 0.985858571                    | TSS1500     | 0.011301146  |
| cg27122725 | 0.191928791 | 0.985858571                    | 5'UTR       | 0.04731291   |
| cg19135245 | 0.244476459 | 0.985858571                    | TSS1500     | 0.005449225  |
| cg01967637 | 0.254982491 | 0.985858571                    | 5'UTR       | −0.002502373 |
| cg21702128 | 0.310632555 | 0.985858571                    | TSS1500     | 0.003068137  |
| cg06968181 | 0.341577022 | 0.985858571                    | TSS1500     | 0.015388067  |
| cg26464411 | 0.354871977 | 0.985858571                    | TSS1500     | 0.018949214  |
| cg14558428 | 0.355258299 | 0.985858571                    | 5'UTR       | 0.000239683  |
| cg00629244 | 0.377208819 | 0.985858571                    | TSS200      | −0.003861647 |
| cg08818984 | 0.399423704 | 0.985858571                    | 1stExon     | 0.000741573  |
| cg23776787 | 0.447963073 | 0.985858571                    | 1stExon     | 0.016867225  |
| cg13648501 | 0.469320108 | 0.985858571                    | 5'UTR       | 0.016976965  |
| cg03906910 | 0.497810362 | 0.985858571                    | 1stExon     | 0.010141716  |
| cg18068240 | 0.512386308 | 0.985858571                    | 5'UTR       | 0.00411793   |
| cg21209684 | 0.572146062 | 0.985858571                    | 5'UTR       | 0.00264155   |

**Table 15** Differential methylation according to baseline depression (BDI-II) (promoter-associated *NR3C1* probes) (Continued)

| CpG        | <i>p</i>    | Adjusted <i>p</i> <sup>a</sup> | Gene region | Δβ            |
|------------|-------------|--------------------------------|-------------|---------------|
| cg16335926 | 0.591526062 | 0.985858571                    | TSS1500     | 0.003497235   |
| cg04111177 | 0.611636013 | 0.985858571                    | 5'UTR       | 0.000989473   |
| cg13764763 | 0.65429048  | 0.985858571                    | TSS1500     | 0.008619545   |
| cg14939152 | 0.795018613 | 0.988065713                    | 5'UTR       | 0.001196214   |
| cg26720913 | 0.798815567 | 0.988185518                    | 1stExon     | 0.029770303   |
| cg18998365 | 0.856200047 | 0.991299612                    | 5'UTR       | 0.016860787   |
| cg07733851 | 0.871021038 | 0.992356236                    | 5'UTR       | 0.022062569   |
| cg18718518 | 0.873514581 | 0.992390392                    | TSS1500     | 0.015863129   |
| cg06952416 | 0.87457104  | 0.992509068                    | 5'UTR       | 0.033670117   |
| cg17860381 | 0.875979533 | 0.992611179                    | 5'UTR       | 0.000759159   |
| cg18019515 | 0.916972995 | 0.995093105                    | TSS200      | 0.000584247   |
| cg11152298 | 0.925587204 | 0.995541393                    | TSS200      | 1.94E-05      |
| cg17342132 | 0.936691926 | 0.996297376                    | Body        | − 0.021376556 |
| cg15645634 | 0.948521192 | 0.997183288                    | 5'UTR       | − 0.001498486 |
| cg24026230 | 0.951640736 | 0.997379305                    | 5'UTR       | 0.002161506   |
| cg10847032 | 0.979982104 | 0.998978654                    | TSS1500     | 0.004273011   |
| cg15910486 | 0.985159251 | 0.999341633                    | 5'UTR       | 0.003121248   |

Δβ = mean β (severely depressed) – mean β (mildly depressed)

TSS transcription start site, UTR untranslated region

<sup>a</sup>Adjusted for multiple testing [45]**Abbreviations**

BAI: Beck Anxiety Inventory; BBB: Beating the Blues before Birth; BDHI: Beck Depression Inventory-II; CBT: Cognitive behavioral therapy; DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, 4th Edition; MDS: Multidimensional scaling; PCA: Principal component analysis; RCT: Randomized controlled trial; TAU: Treatment as usual

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**Availability of data and materials**

The data sets used and/or analyzed during the current study are available from the corresponding author, on reasonable request.

**Authors' contributions**

JM, AG, and CH contributed to the design and implementation of the original randomized controlled trial. RS and AS assisted in the analysis of DNA methylation data. SdR and LB contributed to the collection of the 5-year follow data including the statistical analysis and preparing of the manuscript. TR and HB aided in interpreting the results and writing of the manuscript. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

The original RCT and the current follow-up study were both approved by the Human Research Ethics Committees of Austin Health, Melbourne, Australia. Trial Registration of the original RCT: ACTRN12607000397415. Registered on 2 August 2007, <https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=82169>. Informed consent was given by one of the children's parents at the outset of the study.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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